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(54) Title: MAST CELL PROTEASE PEPTIDE INHIBITORS

(57) Abstract

Compositions and methods for inhibiting a complex containing a mast cell protease are provided. The compositions are useful for treating inflammatory disorders, such as asthma, that are mediated by release of a tryptase–6 protein. Methods for identifying additional specific inhibitors of a complex containing tryptase–6 protein and a serglycin glycosaminoglycan also are provided.

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MAST CELL PROTEASE PEPTIDE INHIBITORS

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Related Applications

This application claims priority under 35 USC § 119(e) from U.S. Provisional Patent Application Serial No. 60/037,090, filed on February 5, 1997, entitled MAST CELL PROTEASE PEPTIDE INHIBITORS. The contents of the provisional application are hereby expressly incorporated by reference.

Field of the Invention

This invention relates to compositions containing a mast cell protease inhibitor and methods for use thereof in the prevention and treatment of inflammatory disorders mediated by mast cell tryptases. Methods utilizing the compositions for identifying additional inhibitors of the mast cell protease also are provided.

Background of the Invention

Mast cells play central roles in varied inflammatory reactions due to their ability to release a diverse array of biologically active factors. During the last decade, the primary focus has been on the role of mast cell-derived histamine, leukotrienes, prostaglandins, cytokines, and chemokines in inflammation. Little attention has been paid to the role of tryptases even though these serine proteases are major constituents of the secretory granules of human, mouse, rat, gerbil, and dog mast cells. Accordingly, the mechanisms by which mast cell tryptases mediate inflammation have not been identified.

All mast cell proteases are targeted to the secretory granule as inactive zymogens but they are rapidly activated at this site. Thus, they are stored in the granule in their mature, enzymatically active forms. Tryptases, the major secretory granule proteases of human mast cells, are glycosylated, heparin-associated tetramers of heterogenous, catalytically active subunits. These enzymes are stored in an enzymatically inactive state in the mast cell's secretory granules and are released from the cell following activation through the high affinity IgE receptor. Tryptases have been implicated in a variety of biological processes including tissue inflammation.

Various attempts have been made to identify inhibitors of tryptase for treating inflammatory disorders. For example, small aromatic molecules have been proposed as tryptase

inhibitors for preventing and treating inflammatory diseases associated with the respiratory tract, such as asthma and allergic rhinitis. (See, e.g., U.S. 5,525,623, issued to Spear et al., "Compositions and Methods for the Treatment of Immunomediated Inflammatory Disorders"; and International Application Nos. PCT/US95/11814, WO96/09297, and PCT/US94/02706, WO94/20527, Applicant: Arris Pharmaceutical Corporation.) Unfortunately, such molecules nonspecifically inhibit a variety of serine proteases (including pancreatic trypsin) that are present *in vivo* and, accordingly, the therapeutic value of such molecules for treating conditions mediated by mast cell tryptase remains questionable.

In view of the demonstrated involvement of mast cells in the initiation of inflammation, a need still exists to understand the mechanisms by which mast cells control such inflammation and to develop new and useful agents that inhibit or prevent inflammation in the first instance. Preferably, such agents would selectively inhibit specific components produced by the mast cell that are responsible for the inflammation, thereby requiring administration of relatively low doses of the agent and minimizing the likelihood of side reactions that may be associated with the administration of a high dosage of the agent.

Summary of the Invention

The present invention overcomes the problems of the prior art by providing a preferred peptide substrate (protease inhibitor) and its derivatives which can be used to selectively inhibit a mast cell tryptase that induces neutrophilia when administered to mice. The invention involves in one respect the discovery of a peptide sequence (SEQ. ID NO.1) that is a substrate for a complex containing mouse mast cell protease 6 ("mMCP-6") and heparin glycosaminoglycan. This peptide sequence can be used to selectively inhibit this and related mast cell tryptase complexes *in vitro* and *in vivo*. Although not intending to be bound to a particular mechanism of action, it is believed that the human tryptases α, I, β/II, and III (GenBank Accession Nos. are shown in the sequence listing) and rat tryptase (GenBank Accession No. U67909, J. Exp. Med. 1997; 185:13-29) are the homologs of mMCP-6 and that one or more of these human tryptases play a key role in the pathogenesis of mast cell-mediated inflammatory disorders including the emigration of neutrophils.

In view of the foregoing, the protease inhibitors of the invention are useful for treating a variety of inflammatory disorders including asthma, allergic rhinitis, urticaria and antioedema, eczematous dermatitis (atopic dermatitis), and anaphylaxis, as well as hyperproliferative skin disease, peptic ulcers, inflammatory bowel disorder, inflammatory skin conditions, and the like.

The protease inhibitors of the invention also are useful in screening assays for identifying additional inhibitors that selectively inhibit tryptase-6 cleavage of a peptide having SEQ. ID NO.1.

It remains to be determined exactly how many tryptases exist in humans. Four human tryptase cDNAs (designated tryptase α, I, β/II, and III) were isolated by two groups of investigators using two different cDNA libraries (Miller et al., J. Clin. Invest. 1989; 84:1188-1195; Miller et al., J. Clin. Invest. 1990; 86:64-870; Vanderslice et al., Proc. Natl. Acad. Sci. USA 1990; 87:3811-3815). Since the isolated human cDNAs encode enzymes that are >90% identical in their overall amino acid sequences, since humans are not inbred, and since the genes and the region of the chromosome where the tryptase genes reside have not yet been sequenced, the actual number of human mast cell tryptase genes is still unknown. There may be one gene in the human possessing multiple alleles or there may be four or more tryptase genes, some of which are nearly identical. Nevertheless, most investigators believe that human tryptase α and β are derived from distinct genes.

In terms of their overall amino acid sequences, mature mMCP-7 and mMCP-6 are 71% identical. Mature mMCP-7 exhibits homologies with human tryptases α, I, β/II, and III of 74%, 76%, 76% and 78%, respectively, whereas mature mMCP-6 exhibits homologies of 73%, 78%, 78% and 78%, respectively. Thus, it is difficult to conclude from their overall amino acid sequences which tryptase is the human homolog of mMCP-6. However, a comparison of the pro-peptides of mMCP-6 (see below) with those of human tryptases α, I, β/II, and III indicate that human tryptase α probably is not the human homolog of mMCP-6. Comparative analysis of the seven loops that Dr. Šali predicts form the substrate binding pocket of each tryptase also indicates that human tryptase α probably is not the human homolog of mMCP-6. However, at present it is not possible to definitively conclude whether the pocket of mMCP-6 is more similar to that in tryptase I, β/II, or III.

Comparison of the Pro-peptides of Mouse and Human Mast Cell Tryptases

Tryptase	Propeptide (and residue number) -10 ... -3 -1 +1
mMCP-7	Ala-Pro-Gly-Pro-Ala-Met-Thr-Arg-Glu-Gly --- Mature enzyme (SEQ ID NO. 25)
mMCP-6	Ala-Pro-Arg-Pro-Ala-Asn-Gln-Arg-Val-Gly --- Mature enzyme (SEQ ID NO. 26)
h tryptase α	Ala-Pro-Val-Gln-Ala-Leu-Gln-Gln-Ala-Gly --- Mature enzyme (SEQ ID NO. 27)
h tryptase I	Ala-Pro-Gly-Gln-Ala-Leu-Gln-Arg-Val-Gly --- Mature enzyme (SEQ ID NO. 28)
h tryptase II/β	Ala-Pro-Gly-Gln-Ala-Leu-Gln-Arg-Val-Gly --- Mature enzyme (SEQ ID NO. 28)
h tryptase III	Ala-Pro-Gly-Gln-Ala-Leu-Gln-Arg-Val-Gly --- Mature enzyme (SEQ ID NO. 28)

As used herein, "tryptase-6", and "mast cell tryptase" are used interchangeably to refer to an enzymatically active serine protease that selectively cleaves a peptide sequence having SEQ. ID NO.1. The preferred tryptase-6 for use in the screening assays of the invention is the mature mMCP-6 tryptase or the corresponding mature human tryptase. The nucleic acid and encoded protein sequence of the mMCP-6 zymogen from BALB/c mice are provided as SEQ. ID NOS.13, 14 and 15, and have been accorded GenBank Accession Nos. M57625 and M57626 (see also Reynolds, et al., J. Biol. Chem. 1991, 266:3847-3853). The GenBank accession numbers and reference citations for these and related mast cell protease nucleic acids and/or proteins are provided in the Sequence Listing. In particular, the Sequence Listing identifies the nucleic acid and encoded protein sequence of the potential human homolog(s) of the mMCP-6 zymogen (SEQ. ID NOS. 16-23). These protein sequences include the sequence of the "mature" tryptase-6 proteins. By "mature", it is meant that the sequence represents the serine protease which is the enzymatically active form of the protein (i.e., the form that associates with heparin glycosaminoglycan to form the tryptase-6 complex that selectively cleaves SEQ. ID NO. 1).

In general, the enzymatically active serine proteases of the invention are associated with a mast cell specific glycosaminoglycan such as heparin in a complex that can be formed *in vitro* and is also known to exist *in vivo*. Surprisingly, association of a glycosaminoglycan, such as heparin glycosaminoglycan, with the tryptase-6 appears to be essential for the peptide substrate specificity of the cleavage reaction. The Examples demonstrate the extraordinary specificity of an mMCP-6 tryptase/heparin glycosaminoglycan complex for cleaving SEQ. ID NO. 1 and the lack of specificity for mMCP-6 in the absence of this glycosaminoglycan. Prior to this discovery, the dependence of mMCP-6 cleavage specificity on an association with heparin glycosaminoglycan was unknown and could not have been predicted in view of the reported nonspecific cleavage properties of this tryptase or its homologs in other species.

According to one aspect of the invention, a mast cell tryptase-6 inhibitor that competitively inhibits cleavage of a peptide having SEQ. ID NO. 1 by a mast cell protease is provided. Preferably, the mast cell tryptase-6 is mMCP-6 or human tryptase that is complexed with a mast cell specific glycosaminoglycan (e.g., heparin or ChS-E glycosaminoglycan). In a particularly preferred embodiment, the mast cell tryptase-6 inhibitor is a peptide having the amino acid sequence: Arg-Asn-Arg-Gln-Lys-Thr (SEQ. ID NO.1). The invention also includes functionally equivalent peptides of SEQ. ID NO. 1, namely, (1) fragments (2) chemically modified forms of the peptide, and (3) homologs of SEQ I.D. No. 1 that can be used in

accordance with the methods of the invention to selectively inhibit a mast cell tryptase-6 complex *in vitro* or *in vivo*. Functionally equivalent peptides contain from three to twelve amino acids and are capable of inhibiting the specific cleavage of SEQ. ID NO. 1 by a mast cell tryptase-6 complex, i.e., tryptase-6 associated with a serglycin proteoglycan.

According to one aspect of the invention, a method for inhibiting a mast cell tryptase-6 complex that selectively cleaves SEQ. ID NO. 1 is provided. The method involves contacting the mast cell tryptase-6 complex with one or more protease inhibitors of the invention for a time sufficient to permit the protease inhibitor to enter the substrate binding site of the enzyme.

According to still another aspect of the invention, a method for selecting a mast cell tryptase-6 complex inhibitor is provided. The method involves determining whether a mast cell tryptase-6 complex cleaves a peptide having SEQ. ID NO. 1 in the presence of a putative protease inhibitor. In a particularly preferred embodiment, the putative protease inhibitor is contained in a phage display library. These methods (also referred to herein as "screening assays") are useful for identifying the above-mentioned functionally equivalent peptides of SEQ. ID NO.1. Such screening assays rely upon biochemical measurements, physical measurements or functional activity tests to determine whether cleavage of SEQ. ID NO. 1 has occurred.

Exemplary functionally equivalent peptide fragments of SEQ. ID NO. 1 are provided in SEQ. ID NOS. 2-11. Exemplary functionally equivalent homologs of SEQ. ID NO. 1 are derived from the naturally-occurring proteins that contain SEQ. ID NO.1 or a sequence that is substantially identical to SEQ. ID NO.1. Functionally equivalent peptides of SEQ. ID NO.1 optionally contain from one to six conservative amino acid substitutions.

The protease inhibitors of the invention competitively inhibit cleavage by a mast cell tryptase-6 of SEQ. ID NO.1. The preferred protease inhibitors of the invention are irreversible competitive inhibitors. Such irreversible protease inhibitors include, for example, a derivatizing agent that reacts with an amino acid in the substrate binding site of the mast cell protease to form a covalent bond. Preferably, such derivatizing agents can reside anywhere in the protease inhibitor. In general, such irreversible protease inhibitors have a structure that mimics the transition state of the enzyme-substrate complex formed during reaction of the mast cell protease with SEQ. ID NO. 1. According to yet other aspects of the invention, pharmaceutical compositions containing the above-described protease inhibitors and methods for making the pharmaceutical compositions are provided. The methods involve placing the protease inhibitors of the invention in a pharmaceutically acceptable carrier.

According to a related aspect of the invention, a method for treating a mast cell-mediated inflammatory disorder is provided. Exemplary mast cell-mediated inflammatory disorders include asthma, allergic rhinitis, urticaria and antioedema, and eczematous dermatitis (atopic dermatitis), and anaphylaxis, as well as hyperproliferative skin disease, peptic ulcers, inflammatory bowel disorder, inflammatory skin conditions, and the like. Such mast cell-mediated inflammatory disorders are believed by the Applicants to be mediated by a tryptase-6. Accordingly, the method of the invention involves administering to a subject in need of such treatment one or more protease inhibitors of the invention in a pharmaceutically acceptable carrier. The protease inhibitor is administered to the subject in an amount effective to inhibit activity of a mast cell tryptase-6 complex in said subject.

These and other aspects of the invention as well as various advantages and utilities will be more apparent with reference to the detailed description of the preferred embodiments and in the accompanying drawings. All patents, patent publications and references identified in this document are incorporated in their entirety herein by reference.

Detailed Description of the Invention

The present invention in one aspect involves the discovery that a macromolecular complex containing mouse mast cell tryptase-6 ("mMCP-6") associated with heparin glycosaminoglycan selectively cleaves a peptide having the sequence of SEQ. ID NO. 1 and that this and other structurally-related peptides can be used to selectively inhibit the enzymatic activity of mMCP-6 and its homologs (e.g., human tryptase) *in vitro* and *in vivo*. Although not intending to be bound to any particular mechanism or theory, it is believed that the naturally-occurring ("physiological") substrate(s) of tryptase *in vivo* contains a peptide sequence that is substantially identical to SEQ. ID NO. 1 and that cleavage by a tryptase-6 *in vivo* of its physiological substrate represents a fundamental step in the pathogenesis of mast cell mediated-inflammatory disorders. By "substantially identical" it is meant that the peptide cleavage site sequence of the physiological substrate of tryptase-6 differs from SEQ. ID NO. 1 by, at most, one amino acid.

As used herein, a "tryptase-6" protein refers to the enzymatically active "mature" mMCP-6 protein, its naturally occurring alleles, and homologs of the foregoing proteins in other species. The tryptase-6 proteins, like other serine proteases, are synthesized in cells as zymogens (i.e., in an enzymatically inactive precursor form) which include a hydrophobic "pre" peptide sequence (also referred to as a "signal sequence" or "signal peptide") and a "pro" sequence (also referred

to as a "pro-peptide sequence") attached to the N-terminal portion of the mature protein. The nucleic acid and encoded protein sequence of the mMCP-6 zymogen from BALB/c mice are provided as SEQ ID NOS. 13, 14 and 15, and have been accorded GenBank Accession Nos. M57625 and M57626, (see also Reynolds, et al., J. Biol. Chem. 1991, 266:3847-3853). The GenBank accession numbers and reference citations for these and other mast cell protease nucleic acids and/or proteins are provided in the Sequence Listing. In particular, the Sequence Listing identifies the nucleic acid and encoded protein sequence of the potential human homologs of the mMCP-6 zymogen (SEQ ID NOS. 16-23), including the protein sequences for the "mature" tryptase-6 proteins for these proteins. By "mature", it is meant that the sequence represents the serine protease which is the enzymatically active form of the protein.

The tryptase-6 proteins that are inhibited by the protease inhibitors of the invention are members of the serine protease superfamily. In particular, the tryptase-6 proteins are members of the trypsin-like serine protease family of proteins that are the major constituents of the secretory granules of mouse, rat, gerbil, dog, and human mast cells. Lung, heart, and skin mast cells in the BALB/c mouse express at least two tryptases [designated mouse mast cell protease 6 ("mMCP-6") and 7 ("mMCP-7")] which are 71% identical in terms of their overall amino acid sequences. This tryptase family of mast cell proteases has been implicated in the pathobiology of FcϵRI-elicited responses in airways. Linkage analysis has implicated the region of chromosome 17 where the mMCP-6 and mMCP-7 genes reside as one of the candidate loci for the inheritance of intrinsic airway hyper responsiveness. A physiological substrate for the mMCP-7 protein recently has been identified as fibrinogen (see, U.S. Serial No. 60/032,354 filed December 4, 1996, now U.S. Serial No.08/978,404 filed November 25, 1997 by R. Stevens). To date, the inability to definitively identify the physiological substrate for mMCP-6 has prevented the development of therapeutic agents that mediate conditions attributable to an under- or over-abundance of the mMCP-6 protein or its physiological substrate. Accordingly, the identification of the specific cleavage sequence disclosed herein for the mMCP-6 protein permits the development of therapeutic agents for treating conditions that are mediated by this tryptase.

The mMCP-6 protein is stored in acidic granules of the cell as a complex containing the mature, enzymatically active form of the enzyme ionically bound to the glycosaminoglycan side chains of serglycin proteoglycans (Ghildyal, et al., J. Exp. Med. 1996; 184:1061-1073, whose content is incorporated herein by reference in its entirety). As used herein, a "tryptase-6 complex" refers to a mature mMCP-6 tryptase (its alleles, homologs) in association with a

serglycin proteoglycan (containing heparin or another mast cell- specific chondroitin). Although mMCP-6 and mMCP-7 are negatively charged at neutral pH and are associated with serglycin proteoglycans at neutral pH, the two tryptases differ in their ability to dissociate from the proteoglycans following their exocytosis from the mast cell. As a result, these proteases exhibit different substrate specificities and are metabolized quite differently in mice undergoing passive systemic anaphylaxis.

Modeling and site-directed mutagenesis analysis of recombinant pro-mMCP-7 (i.e., the expressed protein with its normal "pro-peptide" sequence) suggest that this mature tryptase readily dissociates from serglycin proteoglycans when the protease/proteoglycan macromolecular complex is exocytosed into a pH 7.0 environment because the glycosaminoglycan-binding domain on the surface of mMCP-7 consists primarily of a cluster of His residues. In contrast, the mMCP-6 protein does not readily dissociate from serglycin proteoglycans because its glycosaminoglycan-binding domain consists primarily of a cluster of strongly basic Lys or Arg residues, as found in all mast cell chymases. Although not intending to be limited to a particular mechanism of action, the prolonged retention of exocytosed mMCP-6 complex in the extracellular matrix around activated tissue mast cells is believed by us to be associated with a local activity for this tryptase, whereas the rapid dissipation of mMCP-7 from tissues and its poor ability to be inactivated by circulating protease inhibitors suggests that this distinct, but homologous, tryptase cleaves proteins at more distal sites.

More than 25 genes have been cloned that encode the peptide cores of different proteoglycans. mMCP-6 is preferentially bound to the glycosaminoglycan (GAG) side chains of the serglycin family of proteoglycans. Those mast cells that express mMCP-6 generally have serglycin proteoglycans that have covalently bound heparin chains but sometimes these proteoglycans have highly charged chondroitin sulfate (ChS) chains (e.g., ChS-diB and ChS-E). Human lung mast cells also can express serglycin proteoglycans that can have either heparin or ChS-E chains (Stevens et al., Proc. Natl. Acad. Sci. USA 1988; 85:2284-2287). Although small amounts of serglycin proteoglycan containing ChS-E chains have been identified in cultured human eosinophils (Rothenberg et al., J. Biol. Chem. 1988; 263:13901-13908), mast cells are the only mammalian cell type which can produce relatively large amounts of ChS-E.

It is not known why mast cells synthesize very different types of GAG onto a serglycin peptide core. Since more than 30 enzymes are involved in the differential biosynthesis of heparin and ChS-E, the switch in GAG expression in the mast cell probably is biologically

relevant. It is possible that ChS-E influences the substrate specificity of mMCP-6 differently than heparin. Accordingly, other highly charged GAG, such as ChS-E, also may regulate the substrate specificity of mMCP-6.

The specificity of the mMCP-6 complex for cleaving SEQ. ID NO.1 was discovered during experiments designed to elucidate the preferred amino acid sequences that are cleaved by this mast cell protease. Surprisingly, heparin glycosaminoglycan was found to alter the substrate specificity of mMCP-6 for cleaving peptides in a tryptase-specific bacteriophage display library. We believe that heparin glycosaminoglycan may sterically restrict the substrate-binding cleft of mMCP-6 by directly influencing one of the seven loops that form this pocket. The present invention is based upon the discovery that the mMCP-6 complex selectively cleaves a peptide containing SEQ. ID NO.1 but that this enzymatic activity is not shared with mMCP-6 (in the absence of a serglycin proteoglycan) or with mMCP-7.

A "mast cell protease inhibitor" or a "protease inhibitor", as used herein, refers to a peptide which competitively inhibits cleavage by a tryptase-6 complex of SEQ. ID NO. 1. The protease inhibitors of the invention are peptides that are or contain SEQ. ID NO.1 or its functionally equivalent peptides. Protease inhibitors which are functionally equivalent peptides of SEQ. ID NO.1 are identified in screening assays which measure the ability of a putative protease inhibitor to prevent cleavage by a tryptase-6 complex (e.g., a mMCP-6 or human tryptase-6 complex) of SEQ. ID NO.1 or its functional equivalents.

As used herein, "functionally equivalent peptides" of SEQ. ID NO.1 refer to (1) fragments, (2) chemicallly modified derivatives, and (3) homologs of SEQ. ID NO.1, that can be used in accordance with the methods of the invention to inhibit cleavage by a tryptase-6 complex of SEQ. ID NO.1. Functionally equivalent peptides contain from three to twelve amino acids and competitively inhibit cleavage by a tryptase-6 complex of a peptide that is or that includes SEQ. ID NO.1.

Functionally equivalent peptides of SEQ. ID NO.1 are identified in one or more "screening assays". In general, such screening assays are of two types: (1) binding assays which detect a complex containing the putative protease inhibitors associated with a tryptase-6 complex (e.g., mMCP-6/heparin glycosaminoglycan) and (2) enzymatic activity assays which measure the ability of a putative protease inhibitor to inhibit cleavage by a tryptase-6 complex of SEQ. ID NO.1 or a functionally equivalent peptide of SEQ. ID NO.1. In general, the binding assays (preferably, irreversible binding) involve the detection of a labeled inhibitor (e.g., a fluorescent

or radioactive tag) associated with the tryptase-6 complex; enzymatic assays measure the ability of the putative protease inhibitor to competitively inhibit cleavage by the tryptase-6 complex of SEQ. ID NO.1.

In a particularly preferred embodiment, the protease inhibitor of the invention has SEQ. ID NO.1. This amino acid sequence was identified in a tryptase-specific bacteriophage peptide display library that was screened with mMCP-6 to determine its preferred substrate peptide sequence (see Example). No particular peptide sequence was favored when the library was screened with mMCP-6 alone; however, a phage clone was preferentially obtained when the library was screened with an mMCP-6/heparin complex. Analysis of this clone revealed a sequence (SEQ. ID NO.1) that was susceptible to cleavage by the mMCP-6/heparin complex. A search of GenBank indicated that a sequence that is substantially identical to SEQ. ID No.1 is present in human fibronectin (SEQ. ID NO. 12, amino acid nos. 1351 - 1356). Although not intending to be bound to any particular theory or mechanism, it is believed that this protein represents a physiological substrate of human tryptase-6 and that tryptase-6 mediates the pathogenesis of inflammatory disorders by selectively cleaving fibronectin at an amino acid sequence that is substantially identical to SEQ. ID NO.1. (See Example for a more detailed discussion of the role played by fibronectin in integrin-binding and the implications of this discovery with respect to the role played by tryptase in mast-cell mediated inflammation by controlling integrin-dependent signaling pathways.)

The amino acid sequence of SEQ. ID NO.1 is:

Arg-Asn-Arg-Gln-Lys-Thr (SEQ.ID NO.1).

A generic formula that embraces SEQ. ID NO. 1 is:

R/K-X-R/K-X-R/K-X,

where R/K represents an Arg or Lys (basic amino acids)and X represents a neutral amino acid. It is believed that the highly charged basic character of SEQ. ID NO.1 plays an important role in the localization of the peptide to substrate binding site of the mast cell tryptase-6.

As used herein, functionally equivalent "peptide fragments" of SEQ. ID NO.1 refer to fragments of SEQ. ID NO. 1 that contain from three to five amino acids (SEQ. ID NOS. 2 through 10). Peptide fragments can be synthesized without undue experimentation using standard procedures known to those of ordinary skill in the art. Each of SEQ. ID NOS. 2-10 contains at least one basic amino acid that can serve as a P1 amino acid for cleavage by the mast cell serine protease.

In general, the term "homolog" refers to a molecule that shares a common structural feature with the molecule to which it is deemed to be an homolog. As used herein in reference to the protease inhibitors of the invention, a "functionally equivalent peptide" that is a “homolog" of SEQ. ID NO.1 is a peptide which shares a common structural feature (amino acid sequence homology) and a common functional activity (inhibiting tryptase-6 complex cleavage of SEQ. ID NO.1) with SEQ. ID NO.1. Functionally equivalent peptide homologs of SEQ. ID NO.1 are derived from naturally-occurring proteins that contain an amino acid sequence having sequence homology to SEQ. ID NO.1. Preferably, such homologs contain at least four and, preferably, five of the amino acid residues in the same order as SEQ. ID NO.1 and, optionally, contain from zero to five amino acids that are derived from the naturally-occurring amino acid sequence. Exemplary functionally equivalent peptide homologs of SEQ. ID NO. 1 include amino acids 1351-1356, 1350-1356, 1349-1356, 1348-1356, 1347-1356, 1346-1356, 1351-1357, 1351-1358, 1351-1359, 1351-1360, 1351-1361- and 1346-1361 of fibronectin (SEQ. ID No. 12).

A computer search of a protein database with SEQ. ID NO.1 revealed a substantially identical sequence in fibronectin. Athough not intending to be bound to any particular theory, it is believed that the physiological substrate for mMCP-6 complex is fibronectin and that tryptase-6 complex is capable of selectively cleaving this protein *in vitro* or *in vivo*. Thus, fibronectin represents a “protein homolog" of SEQ. ID NO.1 from which functionally equivalent peptide homologs of SEQ. ID NO. 1 can be derived.

Fibronectin contains the sequence, Arg-Gly-Arg-Gln-Lys-Thr (SEQ. ID NO.11), which differs from SEQ. ID NO.1 in a single amino acid. This sequence is found in fibronectin at amino acids 1351-1356 and is believed to be a cleavage site for the mast cell serine protease *in vivo*. Functionally equivalent peptide homologs of SEQ. ID NO.1 that are derived from fibronectin include from zero to five amino acids that are N-terminal and/or C-terminal to SEQ. ID NO.11 in the this protein homolog. Additional SEQ. ID NO.1 protein homologs having sequence homology with SEQ. ID NO.1 can be identified using art-recognized methods, e.g., searching data bases such as GENBANK for homologous peptides and/or proteins, as new sequences are added to these databases.

Functionally equivalent peptides of SEQ. ID NO.1 optionally contain conservative amino acid substitutions, provided that the peptides which contain the conservative substitutions competitively inhibit SEQ. ID NO.1 binding to, or cleavage by, a mast cell tryptase-6 complex in the above-mentioned screening assays. As used herein, "conservative amino acid substitution"

refers to an amino acid substitution which does not alter the relative charge or size characteristics of the peptide in which the amino acid substitution is made. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M,I,L,V; (b) F,Y,W; (c) K,R,H; (d) A,G; (e) S,T; (f) Q,N; and (g) E,D. In the particularly preferred embodiments, the functionally equivalent peptides of SEQ. ID NO.1 include one or more conservative amino acid substitution in which arginine and lysine are substituted for one another. It is believed that one, two, or three conservative amino acid substitutions can be made in SEQ. ID NO.1 without adversely affecting the ability of the peptide to competitively bind to/inhibit a tryptase-6 complex.

Preferably, the protease inhibitors of the invention are peptides that include one or more inter-amino acid bonds that are non-hydrolyzable *in vivo*. For example, the peptide may contain one or more D-amino acids, thereby rendering the peptide less susceptible to non-specific proteolytic cleavage *in vivo*. Alternatively, or additionally, the peptide may contain a non-hydrolyzable peptide bond. Such non-hydrolyzable peptide bonds and methods for preparing peptides containing same are known in the art. Exemplary non-hydrolyzable bonds include -psi[CH_2NH]- reduced amide peptide bonds, -psi[$COCH_2$]- ketomethylene peptide bonds, -psi[CH(CN)NH]- (cyanomethylene)amino peptide bonds, -psi[CH_2CH(OH)]- hydroxyethylene peptide bonds, -psi[CH_2O]- peptide bonds, and -psi[CH_2S]- thiomethylene peptide bonds. Additional non-hydrolyzable peptide bonds can be identified using no more than routine experimentation.

In the preferred embodiments, a derivatizing agent (X) is covalently coupled to the peptide substrate (protease inhibitor) to form an irreversible protease inhibitor (X-P). Preferably, the derivatizing agent is covalently attached to the N-terminal or the C-terminal amino acid of the protease inhibitor in accordance with standard procedures for derivatizing an amino acid. In general, the derivatizing agent is a reactive group that reacts with an amino acid in the substrate binding site of the mast cell tryptase-6 complex. Preferably, the chemically modified derivative of the peptide substrate (protease inhibitor) possesses a reactive group that functions as an irreversible inhibitor of a tryptase-6 (e.g., mMCP-6). For example, numerous low molecular weight inhibitors of serine proteases have been synthesized that contain a α-fluorinated ketone or α-keto ester derivative of a critical amino acid in the preferred peptide substrate (Angelastro et al., J. Med. Chem. 1990; 33:13-16). Additional exemplary derivatizing agents for conferring upon a peptide substrate the ability to irreversibly bind to the substrate binding site are described

in U.S. 5,543,396, issued to Powers, et al., "Proline Phosphonate Derivative"; and U.S. 5,187,157 and U.S. 5,242,904, issued to Kettner, et al., "Peptide Boronic Acid Inhibitors of Trypsin-Like Proteases".

As discussed above, a computer search of a protein database with SEQ. ID NO.1 revealed that a substantially identical sequence (SEQ. ID NO. 11, fibronectin amino acids 1351-1356) resides in the middle of each subunit of fibronectin. This sequence is conserved from rats to humans. As discussed in detail in the Example, fibronectin possesses numerous conserved domains that enable fibronectin to interact simultaneously with different proteins on the cell's surface and in the extracellular matrix. The mMCP-6 susceptible sequence in fibronectin is located between the collagen and integrin binding domains. Based upon this observation and the results disclosed herein, we believe that specific cleavage at this site has a dramatic effect on fibronectin-mediated adhesion of fibroblasts and inflammation that is mediated by integrin signal transduction.

Described in the Example is an experiment in which mMCP-6 was injected into the peritoneal cavity of a mouse animal model. Surprisingly, the injection of mMCP-6 into the peritoneal cavity of the animal model specifically recruited neutrophils to this site; however, injection of homologous mMCP-7 into the cavity did not have this effect. As discussed in more detail in the Example, we believe that neutrophil emigration in this *in vivo* assay is mediated, in part, by a generated large-sized fragment of fibronectin that lacks its collagen binding domain. Accordingly, the discovery described herein that mMCP-6 (but not mMCP-7) specifically cuts fibronectin between its collagen- and integrin-binding domains has important implications for mast cell-mediated control of fibrosis and inflammation. More specifically, the animal model results presented herein provide evidence that the protease inhibitors disclosed herein are useful for modulating tryptase-6-mediated inflammation by inhibiting specific cleavage by tryptase of its physiological substrate *in vivo*. Although mMCP-6 and mMCP-7 (described in USSN 60/032,354) have different substrate specificities, we believe that both tryptases alter integrin-mediated signaling pathways: mMCP-7 by cleaving fibrinogen and mMCP-6 by cleaving fibronectin. Thus, the results presented herein further suggest that mast cell tryptases play a central role in mast cell-mediated inflammation by controlling different integrin-dependent signaling pathways.

In view of the foregoing, a method for treating a mast cell-mediated inflammatory disorder is provided. The method involves administering to a subject in need of such treatment

the tryptase-6 complex inhibitors of the invention in a pharmaceutically acceptable carrier and in an amount effective to inhibit activity of a tryptase-6 complex in said subject.

As used herein, a "mast cell-mediated inflammatory disorder" refers to those diseases associated with mast cell tryptase-6 release and susceptible to treatment with a tryptase-6 inhibitor such as disclosed herein. Examples of such disorders include diseases of immediate type hypersensitivity such as asthma, allergic rhinitis, urticaria and antioedema, and eczematous dermatitis (atopic dermatitis), and anaphylaxis, as well as hyperproliferative skin disease, peptic ulcers, inflammatory bowel disorder, inflammatory skin condiitons, and the like. "Hyperresponsiveness" refers to late phase bronchoconstriction and airway hyperreactivity associated with chronic asthma. Hyperresponsiveness of asthmatic bronchiolar tissue is believed to result from chronic inflammation reactions, which irritate and damage the epithelium lining the airway wall and promote pathological thickening of the underlying tissue. Thus, the protease inhibitors of the invention are useful for the treatment (prevent, delay the onset of, or ameliorate the symptoms) of immunomediated inflammatory disorders, and particularly with those associated with the respiratory tract, e.g., asthma, and hyperresponsiveness.

The protease inhibitors described above are administered in effective amounts. An effective amount is a dosage of the protease inhibitor sufficient to provide a medically desirable result. The effective amount will vary with the particular condition being treated, the age and physical condition of the subject being treated, the severity of the condition, the duration of the treatment, the nature of the concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practioner. For example, an effective amount for treating asthma would be an amount sufficient to lessen or inhibit one or more clinically recognized symptoms of asthma. Thus, it will be understood that the protease inhibitors of the invention can be used to treat mast-cell mediated inflammatory disorders prophylactically in subjects at risk of developing such inflammatory disorders. As used in the claims, "inhibit" embraces all of the foregoing. Likewise, an effective amount for treating any of the above-noted inflammatory disorders is that amount which can slow or halt altogether the particular symptoms of such disorders. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment.

Generally, doses of active compounds will be from about 0.01mg/kg per day to 1000 mg/kg per day. It is expected that doses in the range of 50-500 mg/kg will be suitable, preferably orally and in one or several administrations per day. Lower doses will result from other forms of

administration, such as intravenous administration. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compounds.

When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptably compositions. Such preparations may routinely contain salt, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts. As used herein, tryptase-6 inhibitor or protease inhibitor means the compounds described above as well as salts thereof.

The tryptase-6 inhibitors may be combined, optionally, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration into a human or other animal. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the molecules of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

The pharmaceutical compositions may contain suitable buffering agents, including: acetic acid in a salt; citric acid in a salt; boric acid in a salt; and phosphoric acid in a salt.

The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the protease inhibitor, which is preferably isotonic with the blood of the recipient. This aqueous preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation

also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

A variety of administration routes are available. The particular mode selected will depend of course, upon the particular drug selected, the severity of the condition being treated and the dosage required for therapeutic efficacy. The methods of the invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of the active compounds without causing clinically unacceptable adverse effects. Such modes of administration include oral, rectal, topical, nasal, interdermal, or parenteral routes. The term "parenteral" includes subcutaneous, intravenous, intramuscular, or infusion. Intravenous or intramuscular routes are not particularly suitable for long-term therapy and prophylaxis. They could, however, be preferred in emergency situations. Oral administration will be preferred for prophylactic treatment because of the convenience to the patient as well as the dosing schedule.

The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the protease inhibitors into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the protease inhibitors into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

For topical applications, the protease inhibitors can be formulated as ointments or creams. Exemplary pharmaceutically acceptable carriers for peptide drugs, described in U.S. 5,211,657, are useful for containing the protease inhibitors of the invention. Exemplary pharmaceutically acceptable carriers for protease inhibitors that are small molecules and, in particular, for aerosol administration, are described in U.S. 5,525,623. Such preparations also are useful for containing the protease inhibitors of the invention. As used herein, the term "aerosol" refers to a gas-borne

suspended phase of the protease inhibitors that is capable of being inhaled into the bronchioles or nasal passages. Such formulations are particularly useful for treating asthma and hyperresponsiveness.

According to another aspect of the invention, the protease inhibitors of the invention are useful as agents for modulating integrin-mediated signal transduction. Thus, the invention advantageously provides mast cell protease inhibitors in a form that can be administered in accordance with art-recognized methods for drug delivery *in vivo*. For example, the protease inhibitors can be formulated into an aerosol or topical pharmaceutic preparation to deliver to local cells an amount of protease inhibitor sufficient to inhibit mast cell-mediated fibrosis, inflammation, and integrin-related signal transduction pathways such as those involved in cell trafficking and proliferation. Topical application to the skin of a protease inhibitor of the invention is useful for inhibiting cell proliferation associated with conditions such as psoriasis. Aerosol application of a protease inhibitor is useful for inhibiting inflammation associated with asthma and other disorders associated with intrinsic airway hyperresponsiveness.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the protease inhibitors. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the protease inhibitors described above, increasing convenience to the subject and the physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer based systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono- di- and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the protease inhibitor is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,667,014, 4,748,034 and 5,239,660 and (b) diffusional systems in which an active component

permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,832,253, and 3,854,480. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

Use of a long-term sustained release implant may be particularly suitable for treatment of chronic conditions. Long-term release, as used herein, means that the implant is constructed and arranged to delivery therapeutic levels of the active ingredient for at least 30 days, and preferably 60 days. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

According to yet another aspect of the invention, a method for manufacturing a pharmaceutical composition containing the protease inhibitors of the invention is provided. The method involves placing the above-described protease inhibitor in a pharmaceutically acceptable carrier to form a pharmaceutical composition and administering the pharmaceutical composition containing a therapeutically effective amount of the protease inhibitor to the recipient.

It should be understood that the preceding is merely a detailed description of preferred embodiments. It therefore should be apparent to those of ordinary skill in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. All references, patents and patent publications that are identified in this application are incorporated in their entirety herein by reference. The specific example presented below is illustrative only and is not intended to limit the scope of the invention described herein.

Example

Experimental Procedures[1]

Expression of pro-mMCP-6 and pro-EK-mMCP-6-FLAG in Insect Cells -- The novel bioengineering approach developed recently to obtain a pseudozymogen form of mMCP-7 that could be proteolytically activated after its purification from the conditioned media of insect cells was used to obtain a similar pseudozymogen (pro-EK-mMCP-6-FLAG) form of mMCP-6. Expressed pro-EK-mMCP-6-FLAG has an EK-susceptible peptide (Asp-Asp-Asp-Asp-Lys, SEQ ID NO. 29) in between the domain that encodes the endogenous pro-peptide and the N-terminal Ile residue of the mature tryptase. The recombinant protein also has the 8-residue FLAG peptide attached to its C terminus. In order for a serine protease to have catalytic activity the α-amino of

[1]The abbreviations used are: 3D, three dimensional; EK, enterokinase; FLAG, the peptide whose amino acid is Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys, SEQ ID NO. 30; and mMCP, mouse mast cell protease.

the N-terminal Ile residue must form a internal ion pair with the carboxyl group of an internal specific Asp residue after the pro-peptide is removed (Freer et al., Biochemistry 1977, 9:1997-2009). Thus, it is critical that mature mMCP-6 have an N-terminal Ile residue. Because EK is a highly specific enzyme that cleaves the Lys-Ile bond in its recognition motif (Light and Janska, Trends Biochem Sci 1989, 14(3):110-112), is a relatively stable enzyme at pH 5.0, and will specifically cleave pro-mMCP-7-FLAG, it was anticipated that pro-mMCP-6-FLAG could be proteolytically activated by EK under conditions where the recombinant tryptase, itself, would have very little enzymatic activity until the pH is raised to 7.0. That the pseudozymogen also has the FLAG peptide at its C-terminus enabled its rapid purification from the insect cell conditioned media by means of an affinity column containing anti-FLAG IgG antibody (Prickett et al., Biotechniques 1989, 7:580-589; Brizzard et al., Biotechniques 1994, 16:730-735).

While, in theory, EK digestion of pro-mMCP-6-FLAG should remove the modified pro-peptide, the resulting recombinant product still will have the FLAG peptide attached to its C-terminus. Nevertheless, it was anticipated that mMCP-6-FLAG would be enzymatically active because the FLAG peptide does not influence the enzymatic activity of mMCP-7.

The relevant cDNA constructs, created using standard polymerase chain reaction approaches, were inserted in the correct orientation into the multiple cloning site of pVL1393 (PharMingen, San Diego, CA) downstream of the promoter of the polyhedrin gene. Insect cells were induced to express pro-mMCP-6 and pro-mMCP-6-FLAG, as described previously for pro-mMCP-7 (Matsumoto et al., J. Biol. Chem. 1995, 270:19524-19531) and pro-mMCP-7-FLAG. Briefly, purified plasmid DNA (~5 μg) was mixed with 0.5 μg of linearized BaculoGold™ DNA (PharMingen) and calcium phosphate, each resulting DNA solution was added to 3 x 10^6 adherent Spodoptera frugiperda 9 insect cells (Invitrogen, San Diego, CA) that were in their log phase of growth, and the infected cells were cultured for 7 days at 27°C in medium (Invitrogen) supplemented with 10% heat-inactivated fetal calf serum (Sigma, St. Louis, MO). Recombinant virus particles (≥3 x 10^7) from these insect cells were added to a new culture dish containing 6 x 10^6 Trichoplusia ni High Five™ insect cells (Invitrogen) in their log phase of growth, and the infected cells were cultured in serum-free, Xpress medium (BioWhittaker, Walkersville, MD). Generally 4 d later, the conditioned medium was centrifuged at 1500 *g* for 15-min at room temperature before attempting to purify the secreted recombinant protein.

Purification of pro-mMCP-6 and pro-EK-mMCP-6-FLAG from Insect Cell Conditioned Media and EK Activation of the Recombinant Zymogen -- Pro-mMCP-6 and pro-

EK-mMCP-6-FLAG were purified by heparin-Sepharose chromatography, as described for pro-mMCP-7 (Matsumoto et al., J. Biol. Chem. 1995, 270:19524-19531). The purification of pro-EK-mMCP-6-FLAG also was carried out using an affinity column containing the mouse anti-FLAG M2 monoclonal antibody (Eastman Kodak/International Biotechnol.). This immuno-affinity column (2 ml) was washed with 0.1 M glycine, pH 3.5, and then with 50 mM Tris-HCl and 150 mM NaCl, pH 7.4. After the application of the insect cell conditioned media, the column was washed briefly with the above pH 7.4 buffer, and then bound pro-EK-mMCP-6-FLAG was eluted with 0.1 M glycine, pH 3.5. The eluate was collected into tubes that contained 0.1 M Tris-HCl, pH 7.0, to minimize acid-mediated denaturation of pro-EK-mMCP-6-FLAG. The protein concentration of the eluate was estimated by measuring the absorbance at 280 nm.

Purified pro-EK-mMCP-6-FLAG (~100 μg in 100 μl) was separately mixed with 100 μl of a pH 5.2 buffer consisting of 50 mM sodium acetate and 5 mM calcium chloride. One μl of a solution containing 550 U of calf intestine EK (Biozyme) was added to each, and the mixture was incubated at 37°C generally for 3 h to allow EK to activate the zymogen in the absence of heparin. The spectrophotometric method of Svendsen and coworkers (Throm. Res. 1972, 1:267-278) was used to determine whether or not mMCP-6-FLAG is enzymatically active. Generally, 1-μl samples of each activation reaction were placed in 1 ml of a pH 7.4 buffer containing 25 mM sodium phosphate, 1 mM EDTA, and 50 μg of tosyl-Gly-Pro-Lys-p-nitroanilide. The change in optical density at 405 nm was then determined after a 3-min incubation at room temperature. The ability of recombinant mMCP-6-FLAG to cleave the trypsin-susceptible substrates tosyl-Gly-Pro-Arg-p-nitroanilide, benzoyl-Ile-Glu-Gly-Arg-p-nitroanilide, benzoyl-Pro-Phe-Arg-p-nitroanilide, and acetyl-Ile-Glu-Ala-Arg-p-nitroanilide were also evaluated.

SDS-PAGE/Immunoblotting and N-terminal Amino Acid Analysis -- Insect conditioned media (~20 μl) containing either pro-mMCP-6, pro-EK-mMCP-6-FLAG, or EK-activated mMCP-6-FLAG (~1 μl) were diluted in SDS-PAGE buffer (1% SDS, 5% 2-ME, 0.1% bromophenol blue, and 500 mM Tris-HCl, pH 6.8) and boiled for 5 min before being loaded onto 12% polyacrylamide gels. After SDS-PAGE, the resolved proteins were stained with Coomassie Blue or were transferred in 20 mM Tris-HCl, 150 mM glycine, pH 8.3 buffer containing 20% methanol for 2 to 4 h at 200 mA to PVDF membranes (Millipore) using a BIO-RAD (Richmond, CA) immunoblotting apparatus. For immunoanalysis of the resulting protein blots, each membrane was sequentially incubated 1 h in 5% non-fat milk, 1 h with a 1:500 dilution of

affinity-purified rabbit anti-mMCP-6 Ig (Ghildyal et al., J. Immunol. 1994, 153:2624-2630) in TBST buffer (Tris-buffered saline with 0.01% Tween 20), TBST buffer alone, and then a 1:1,000 dilution of anti-rabbit IgG alkaline phosphatase conjugate (~1 ng/ml final concentration) in TBST buffer. Immunoreactive proteins were visualized using nitroblue tetrazolium (0.2 mg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (0.1 mg/ml) as substrates.

For N-terminal amino acid analysis, SDS-PAGE-resolved proteins were electrobloted unto PVDF membranes, briefly stained with 0.5% Ponceau S red (Sigma), and the relevant protein/peptide bands were subjected to automated Edman degradation by the Harvard Microchemistry Facility (Harvard Biological Laboratories, Cambridge, MA).

Screening of a Tryptase-Specific, Bacteriophage Peptide Display Library with mMCP-6 -- A peptide display library that encodes an altered pIII containing at its N terminus the FLAG peptide followed by an 8-residue hypervariable peptide was screened with recombinant mMCP-6-FLAG. Briefly, phage were obtained that express on their surface a pIII fusion protein with an extension peptide consisting of the FLAG peptide and a hypervariable octamer peptide containing a Lys/Arg residue at the P1 site. After the varied phages in the library were allowed to bind to the anti-FLAG IgG column, the immuno-affinity column was incubated with recombinant mMCP-6-FLAG in the presence or absence of heparin. Those phage recovered in the column's eluate were amplified, and the selection procedure was repeated one to three times. By determining the nucleotide sequence of the relevant portion of the geneIII construct in each clone, the amino acid sequence of the mMCP-6-susceptible peptide in the random domain of the pIII fusion protein was deduced. To prepare the phage column used in the screening process, 10 ml of the phage-enriched supernatant was added to 2 ml of 20% polyethylene glycol (8 kDa; Sigma) and 2.5 M NaCl and the mixture incubated at 4°C for 30 min. After a 30 min centrifugation at 10,000 *g*, the recombinant phage in the pellet were resuspended in 2 ml of 150 mM NaCl, 1 mM $CaCl_2$, and 10 mM sodium phosphate, pH 7.0, and applied to a 1-ml affinity column containing the anti-FLAG M1 monoclonal antibody. The column was washed 3 times with 10 ml of the same pH 7.0 buffer to remove unbound phage. EK-activated mMCP-6-FLAG (~50 μg in 200 μl buffer) in the absence or presence of heparin glycosaminoglycan (~50 μg) was added, and the column was sealed and incubated at room temperature for 90 min. After protease treatment, the column was washed with 2 ml of the pH 7.0 buffer to recover those phage which possessed protease-susceptible pIII fusion proteins. Log-phase *E. coli* were infected with the obtained phage to produce phagemid. Bacteria were

again grown in 2x YT medium containing 2% glucose and the phagemid in the bacteria were converted to phage with the addition of helper phage. This screening procedure was repeated up to 4 times to select the phage in the library which are most susceptible to degradation by mMCP-6-FLAG.

E. coli was infected with resulting mMCP-6-FLAG-susceptible phage to generate phagemids. The infected bacteria were seeded onto a plate containing 1.5% agar, 2% Bacto-tryptone, 0.5% Bacto-yeast extract, 2% glucose, 0.09 M NaCl, 0.01 M $MgCl_2$, and 100 μg/ml ampicillin. Individual clones were isolated and grown overnight at 37°C in 2 ml of 2x YT medium containing 2% glucose with 50 μg/ml ampicillin. One ml of the overnight cultures were centrifuged at ~12,000 *g* for 5 min. The bacteria in the pellets were lysed and the DNAs were extracted with mini-prep method. The DNAs were digested with *NotI* and *EcoRI* restriction enzymes at 37^{0}C overnight. The digested DNA mini-preps were subjected to electrophoresis on a 1% agarose gel, and those individual phage clones with ~1300-bp inserts were selected for maxi-preparation of their DNAs using nucleobond DNA-binding columns (The Nest Group). The nucleotide sequences which encode the 8-mer, protease-susceptible peptide domains in the fusion proteins were determined.

In Vitro Degradation of Fibronectin by Recombinant mMCP-6-FLAG -- Five μg of purified mouse fibronectin (Alexis) was suspended in 1 mM EDTA and 25 mM sodium phosphate, pH 7.4, containing 0.01 U EK, 0.5 μg recombinant pro-EK-mMCP-6-FLAG, 0.5 μg recombinant mMCP-6-FLAG (activated with 0.01 U EK), or 0.5 μg recombinant mMCP-7-FLAG (activated with 0.01 U EK). After an incubated for various lengths of times, the resulting digests were subjected to SDS-PAGE. In one experiment, the N-terminal amino acid sequences of the major fibronectin fragments in the mMCP-FLAG digest were determined.

mMCP-6-FLAG-Induced Emigration of Neutrophils Into the Peritoneal Cavity and mMCP-6-Induced Growth of Fibroblasts and their Adhesion to Fibronectin --This experiment is discussed below.--

<u>Results and Discussion</u>

Generation of pro-mMCP-6 and pro-EK-mMCP-6-FLAG in Insect Cells, and EK Conversion of the Recombinant Pseudozymogen to Enzymatically Active Tryptase -- Insect cells infected with the relevant construct secreted large amounts of pro-mMCP-6 and pro-EK-mMCP-6-FLAG into the conditioned media. Based on its deduced amino acid sequence, mMCP-6 has an overall net charge at pH 7.0 that is considerably more negative than any mouse mast cell

chymase (Šali et al., J. Biol. Chem. 1993, 268:9023-9034). Nevertheless, because mMCP-6 does not dissociate easily from its serglycin proteoglycan, it is retained for >1 h in inflammatory sites (Ghildyal et al., J. Exp. Med. 1996, 184:1061-1073). Modeling studies suggested that this unexpected feature of mMCP-6 is caused by an Arg/Lys rich domain that forms on the surface when the tryptase is properly folded. Like pro-mMCP-6, pro-EK-mMCP-6-FLAG bound to a heparin-Sepharose column that had been equilibrated in 100 mM NaCl/10 mM sodium phosphate, pH 5.5. Because both recombinant proteins dissociated from the heparin-Sepharose affinity column when the NaCl concentration of the buffer was raised to >300 mM, it was concluded that the secreted mMCP-6 pseudozymogen is properly folded. Pro-EK-mMCP-6-FLAG also could be readily purified using the immunoaffinity column.

As assessed by SDS-PAGE, the recombinant pseudozymogen decreased ~2 kDa in size when incubated for 3 to 24 h with EK. Amino acid sequence analysis revealed that the resulting product possessed an N-terminal sequence of **X-Y-Z** which is identical to that of mature mMCP-6 deduced from its cDNA (Reynolds et al., J. Biol. Chem. 1991, 266:3847-3853). While recombinant and native mMCP-7 exhibit good catalytic activity in the absence of heparin (Ghildyal et al., J. Exp. Med. 1996, 184:1061-1073), it has been reported that human mast cell tryptases purified from the lung do not exhibit substantial enzymatic activity unless this glycosaminoglycan is present in the assay (Schwartz and Bradford, J. Biol. Chem. 1986, 261:7372-7379; Alter et al., Biochem. J. 1987, 248:821-827). The ability to purify pro-EK-mMCP-6-FLAG from the conditioned media by means of the immuno-affinity column allowed us to determine if the recombinant protease exhibits enzymatic activity in the absence of heparin. Recombinant mMCP-6-FLAG exhibited optimal enzymatic activity at ~pH 7.4 and good enzymatic activity after a 3-h incubation with EK at 37°C at pH 5.2.

The finding that the EK-activated tryptase readily cleaves tosyl-Gly-Pro-Lys-p-nitroanilide and tosyl-Gly-Pro-Arg-p-nitroanilide in the absence of heparin, indicates that the broad catalytic activity of this tryptase is not dependent on heparin-containing serglycin proteoglycans. However, the observation that mMCP-6-FLAG in the presence or absence of heparin does not effectively cleave benzoyl-Ile-Glu-Gly-Arg-p-nitroanilide, benzoyl-Pro-Phe-Arg-p-nitroanilide, or acetyl-Ile-Glu-Ala-Arg-p-nitroanilide indicates that mMCP-6 has a more restricted substrate specificity than trypsin. Models of the three-dimensional (3D) structures of mMCP-6 and mMCP-7 (Matsumoto et al., J. Biol. Chem. 1995, 270:19524-19531; Ghildyal et al., J. Exp. Med. 1996, 184:1061-1073) based on the crystallographic structure of bovine

pancreatic trypsin suggests that seven loops form the substrate-binding cleft of each tryptase, as occurs for other serine proteases (Perona and Craik, Protein Sci. 1995; 4:2337-360). Relative to trypsin, 3 of the 7 loops in mMCP-7 have insertions that make its substrate-binding cleft deeper and more restricted than that of trypsin. Because similar insertions are found in the corresponding loops of mMCP-6, it is not surprisingly that this latter serine protease also has a restricted substrate specificity.

mMCP-6-Induced Emigration of Neutrophils Into the Peritoneal Cavity of BALB/c Mice -- The mast cells that reside in the peritoneal cavity of BALB/c mice express mMCP-6 but not mMCP-7 (Stevens et al., Proc. Natl. Acad. Sci. USA 1994, 91:128-132). Because this observation suggests that mMCP-6, but not mMCP-7, cleaves specific proteins that reside in the peritoneal cavity, enzymatically active mMCP-6-FLAG was injected into the peritoneal cavity to assess whether or not the tryptase can induce an inflammatory reaction. Six to 36 h after mMCP-6-FLAG administration, a pronounced influx of neutrophils was observed in the peritoneal cavity. As typically seen in acute inflammatory responses (Robbins et al., "Inflammation and repair" in *Pathologic Basis of Disease.* 1994, 5th ed., W. B. Saunders Co., Philadelphia, PA, pp 57-60), large numbers of eosinophils, lymphocytes, erythrocytes, basophils, and platelets, were not detected in the peritoneal exudate of the treated mice. However, unlike a typical inflammatory response where monocytes and eosinophils predominant at subsequent time points (Robbins et al., *supra*), kinetic experiments revealed that the mMCP-6-induced neutrophilia persisted for at least 3 days. Thus, the direct or indirect chemotaxis activity of mMCP-6 is relatively neutrophil specific. It also appears that tryptase treatment results in a relatively persistent recruitment of neutrophils into the peritoneal cavity. The observation that pro-mMCP-6-FLAG does not induce neutrophil emigration at the 36 h time point indicates that the induced inflammatory reaction is dependent on enzymatically active mMCP-6. Moreover, the observation that enzymatically active mMCP-7-FLAG 7-FLAG has very little, if any, neutrophil chemotaxis activity in this *in vivo* assay also documents the exquisite specificity of the tryptase effect.

Screening of a Tryptase-Specific Phage Display Peptide Library with Recombinant mMCP-6-FLAG -- The observation that recombinant mMCP-7-FLAG cleaves acetyl-Ile-Glu-Ala-Arg-p-nitroanilide much better than mMCP-6-FLAG *in vitro* and that mMCP-6-FLAG selectively induces neutrophil emigration *in vivo* indicates that the two mouse tryptases have different substrate specificities even though their overall amino acid sequences are quite similar.

Thus, the tryptase-specific, phage peptide display library that helped us identify a physiologic substrate of mMCP-7 (Huang, et al., J Biol Chem. 1997, 272:31885-31893) was used to identify mMCP-6-preferred peptide substrates. When the library was subjected to 4 rounds of treatment with enzymatically-active mMCP-6-FLAG in the absence of heparin glycosaminoglycan, no specific peptide sequence in the hypervariable domain of the pIII fusion protein was obtained in the 30 arbitrarily selected clones (Table I). Nevertheless, the observation that only one of these mMCP-6-susceptible clones had the preferred mMCP-7-susceptible sequence in its pIII fusion protein (Huang, et al., J Biol Chem. 1997, 272:31885-31893) was further evidence that the two homologous tryptases degrade very different substrates. Another family of serine protease genes is present on chromosome 14 that encode cathepsin G (Heusel et al., Blood 1993, 81:614-1623), at least 5 granzymes (Burnet et al., Nature 1986, 322:268-271; Pham et al., Proc. Natl. Acad. Sci. USA 1996, 93:13090-13095), and at least 6 mast cell chymases (Gurish et al., J Biol Chem. 1993, 268:11372-11379; Hunt et al., J. Biol. Chem. 1995, 271:2851-2855). The observation that the two mouse tryptases are very similar in their overall primary sequences but very different in their preferred peptide substrates is further support that the chromosome 14 and chromosome 17 complexes of serine protease genes evolved so that mast cells and other hematopoietic effector cells that express varied members of the two families of serine proteases degrade different panels of proteins.

TABLE I

mMCP-6-susceptible peptides obtained in the absence of heparin

The tryptase-specific, phage peptide display library was incubated 4 times with recombinant mMCP-6-FLAG in the absence of heparin. Clones were isolated and the deduced amino acid sequences of the peptides found in protease-susceptible domains of the pIII fusion protein were deduced.

Clones	Amino Acid Sequence of Peptide
2	Val-Arg-Pro-Val-Lys-Ser-Phe-Arg (SEQ. ID NO. 31)
1	Ser-Leu-Ser-Ser-Arg-Gln-Ser-Pro (SEQ. ID NO. 32)
1	Ser-Pro-Arg-Pro-Arg-Ser-Thr-Pro (SEQ. ID NO. 33)
1	Gln-Arg-Thr-Lys-Arg-Lys-His-Asn (SEQ. ID NO. 34)
1	Gly-Pro-Arg-Leu-Arg-His-Pro-Arg (SEQ. ID NO. 35)
1	Asn-Leu-Arg-Lys-Arg-Lys-Ile-Lys (SEQ. ID NO. 36)
1	Asn-Ser-Thr-Val-Arg-Lys-Arg-Lys (SEQ. ID NO. 37)
1	Pro-Pro-Pro-Phe-Arg-Arg-Ser-Ser (SEQ. ID NO. 38)
1	Pro-Leu-Ile-Leu-Arg-Ser-Arg-Ala (SEQ. ID NO. 39)
1	Lys-Lys-Ile-Glu-Arg-Arg-Asn-Thr (SEQ. ID NO. 40)

1	Gln-Lys-Arg-Gly-Arg-Glu-Pro-Arg (SEQ. ID NO. 41)
1	Glu-Glu-Lys-Lys-Lys-His-Lys-Lys (SEQ. ID NO. 42)
1	Arg-Gln-Asn-Arg-Arg-Pro-Ser-Asn (SEQ. ID NO. 43)
1	Val-Arg-Pro-Ala-Arg-Ala-Leu-His (SEQ. ID NO. 44)
1	Leu-Ile-Ala-Leu-Arg-Ser-Thr-Thr (SEQ. ID NO. 45)
1	Pro-Thr-Pro-Leu-Lys-His-Pro-Arg (SEQ. ID NO. 46)
1	Pro-Tyr-Pro-Pro-Lys-Arg-Thr-Pro (SEQ. ID NO. 47)
1	Leu-Ser-Thr-Ser-Arg-Ala-Ser-Ile (SEQ. ID NO. 48)
1	Thr-Gly-Val-His-Lys-Pro-Ser-Thr (SEQ. ID NO. 49)
1	Leu-Cys-Ala-Lys-Arg-Leu-Tyr-Arg (SEQ. ID NO. 50)
1	Arg-Lys-Pro-Thr-Lys-Lys-Asn-Ser (SEQ. ID NO. 51)
1	Glu-Cys-Arg-Gln-Arg-His-Thr-Arg (SEQ. ID NO. 52)
1	Ser-Leu-Ala-Leu-Arg-Val-Trp-Arg (SEQ. ID NO. 53)
1	Gly-Pro-Arg-Leu-Arg-His-Pro-Arg (SEQ. ID NO. 54)
1	Phe-Ile-Ser-Arg-Arg-Val-Cys-Arg (SEQ. ID NO. 55)
1	Pro-Asp-Asn-Gln-Arg-Tyr-Ile-Thr (SEQ. ID NO. 56)
1	Pro-Leu-Pro-Cys-Lys-Leu-Asp-Ala (SEQ. ID NO. 57)
1	Ile-Arg-Phe-Ala-Arg-Ser-Gln-Ala (SEQ. ID NO. 58)
1	Pro-Thr-Pro-Leu-Lys-His-Pro-Arg (SEQ. ID NO. 59)

The two most prominent features of the peptides obtained by screening the library with mMCP-6-FLAG alone were the over and under representation of positively and negatively charged residues, respectively. One half of the selected clones had 3 or more Lys and/or Arg residues in the susceptible peptide, and 2 of the clones actually had 5 positively charged residues. These findings are consistent with the electrostatic properties of the mMCP-6 model which revealed that the substrate-binding pocket of mMCP-6 is more negatively charged than that in mMCP-7 (Ghildyal et al., J. Exp. Med. 1996, 184:1061-1073). The difference in the electrostatic potential of the pocket is due primarily to loop 3 which has a -3 net charge in mMCP-6 and a 0 net charge in mMCP-7.

When the phage peptide display library was subjected to 2 to 4 rounds of treatment with mMCP-6-FLAG in the presence of an equal amount of heparin glycosaminoglycan, a more limited number of sequences were obtained (Table II). Surprisingly, the 2 clones that were obtained repeatedly had dissimilar sequences of Thr-Pro-Leu-Leu-Lys-Ser-Trp-Leu (SEQ. ID NO. 64) and Arg-Asn-Arg-Gln-Lys-Thr-Asn-Asn (SEQ. ID NO. 65). The latter favored peptides and the other less favored peptides obtained in this selection process were similar in that each had a Pro residue, at least one Thr or Ser residue, and a net charge of only +1 or +2. The discovery that the favored peptide in this series had a Pro residue at its P4 site is of interest because Cromlish and coworkers (1987) found that a human mast cell tryptase purified from the

pituitary will cleave three prohormones *ex vivo* that have Pro residues at their P4 sites and Lys/Arg residues at their P1 sites.

TABLE II
mMCP-6-susceptible peptides obtained in the presence of heparin

The tryptase-specific, phage peptide display library was incubated 2 (A) or 4 (B) times with recombinant mMCP-6-FLAG in the presence of an equal weight amount of heparin. Clones were isolated and the deduced amino acid sequences of the peptides found in protease-susceptible domains of the pIII fusion protein were deduced.

A. Two Rounds of Treatment

Clones	Amino Acid Sequence of Peptide
1	Pro-Phe-Thr-His-Lys-Ser-Leu-Ser (SEQ. ID NO. 60)
1	Ser-Val-Leu-Pro-Lys-Leu-Arg-Ile (SEQ. ID NO. 61)
1	Pro-Lys-Glu-Thr-Lys-Gln-Thr-Asn (SEQ. ID NO. 62)
3	Ser-Leu-Ser-Ser-Arg-Gln-Ser-Pro (SEQ. ID NO. 63)
5	Thr-Pro-Leu-Leu-Lys-Ser-Trp-Leu (SEQ. ID NO. 64)
11	Arg-Asn-Arg-Gln-Lys-Thr-Asn-Asn (SEQ. ID NO. 65)

B. Four Rounds of Treatment

Clones	Amino Acid Sequence of Peptide
1	Pro-Lys-Glu-Thr-Lys-Gln-Thr-Asn (SEQ. ID NO. 62)
1	Ser-Val-Leu-Pro-Lys-Leu-Arg-Ile (SEQ. ID NO. 61)
2	Ser-Leu-Ser-Ser-Arg-Gln-Ser-Pro (SEQ. ID NO. 63)
4	Arg-Asn-Arg-Gln-Lys-Thr-Asn-Asn (SEQ. ID NO. 65)
7	Thr-Pro-Leu-Leu-Lys-Ser-Trp-Leu (SEQ. ID NO. 64)

Despite these interesting findings, we speculated that the favored peptide from the phage display library which possesses a +3 charge probably is more physiologically relevant because its overall charge is similar to that generally obtained when the library was screened with mMCP-6 alone. Why only one +3 positively charged peptide was obtained and why this peptide was not present in the original 30 clones isolated when the library was screened with mMCP-6 alone remains to be determined experimentally. However, the electrostatic potential of the 3D model of mMCP-6 suggests that the putative heparin-binding domain on the surface of this tryptase resides closer to its active site than in all other mMCPs. Thus, it is likely that heparin sterically restricts the substrate-binding cleft of mMCP-6 by directly influencing one of

the 7 loops that form the pocket. The discovery that the substrate specificity of a rat mast cell chymase is also altered by heparin (Le Trong et al., Proc. Natl. Acad. Sci. USA 1987, 84:364-367) now emphasizes the importance of serglycin proteoglycans in fine tuning the substrate specificities of certain members of the chromosome 14 and chromosome 17 families of serine proteases.

A computer search of a protein database with the sequence Arg-Asn-Arg-Gln-Lys-Thr (SEQ. ID NO. 1) present in the positively charged peptide revealed that a nearly identical sequence (i.e., Arg-Gly-Arg-Gln-Lys-Thr, SEQ. ID NO. 11) resides in the middle of each subunit of fibronectin and that this sequence is conserved from rats to humans. Fibronectin is an abundant protein in plasma and varied extracellular matrices and plays a central role in cellular adhesion. This adhesion protein is a dimer consisting of ~220-kDa polypeptides that are disulfide bonded at the C terminus (Kornblihtt et al., EMBO J. 1985, 4:1755-1759; Skorstengaard et al., Eur. J. Biochem. 1986, 161:441-453). Its primary structure can vary somewhat due to differential splicing of the transcript but each subunit consists of nearly 2400 residues. These subunits possess numerous conserved domains that enable fibronectin to interact simultaneously with different proteins on the cell's surface and in the extracellular matrix. For example, a domain near the N-terminus binds to varied native and denatured collagens, whereas the C-terminal half of the fibronectin contains adjacent domains that allow fibronectin to interact simultaneous with varied integrins and proteoglycans on the surface of the cell. In the case of fibroblasts, fibronectin forms focal adhesions with β_1 integrins and syndecan proteoglycans thereby inducing synergistic signaling through distinct pathways (Woods and Couchman, Mol. Biol. Cell 1994, 5:183-192; Couchman and Woods, J. Cell. Biochem. 1996, 61:578-584). The *in vitro* adhesion of melanoma cells to fibronectin is also mediated by the cooperative action of β_1 integrins and cell surface proteoglycans (Iida et al., J. Cell Biol. 1992, 118:431-444; Wahl et al., J. Leukocyte Biol. 1996, 59:789-796). The mMCP-6-susceptible sequence in fibronectin is at residues 1351 to 1356 between the collagen and integrin binding domains. Thus, the specific cleavage at this site should have a dramatic effect on the fibronectin-mediated adhesion of fibroblasts.

In Vitro Digestion of Fibronectin and Disruption of Fibronectin-Mediated Adhesion of Fibroblasts by mMCP-6-FLAG -- Fibronectin was readily cleaved by the mMCP-6-FLAG/heparin complex *in vitro* but not by mMCP-7-FLAG either in the presence or absence of heparin. Fibronectin is susceptible to cleavage by a wide range of neutral proteases, including

chymotrypsin (Ehrismann et al., J. Biol. Chem. 1982, 257:7381-7387), trypsin (Mosher and Proctor, Science 1980, 209:927-929), α-thrombin (Furie and Rifkin, J. Biol. Chem. 1980, 255:3134-3140), plasmin (Jilek and Hörmann, Hoppe-Seyler's Z. Physiol. Chem. 1977, 358:133-136), plasminogen activator (Quigley et al., Proc. Natl. Acad. Sci. USA 1987, 84:2776-2780), cathepsin G (Vartio et al., J. Biol. Chem. 1981, 256:471-477), urokinase (Gold et al., Biochem. J. 1989, 262:529-534), elastase (McDonald and Kelley, J. Biol. Chem. 1980 255:8848-8858), and mast cell chymases (Vartio et al., J. Biol. Chem. 1981, 256:471-477). Because of its exquisite protease-susceptibility, fibronectin is routinely used to assess general neutral protease activities in samples. BALB/c mouse bone marrow-derived mast cells, developed *in vitro* using T cell-conditioned media, possess serine proteases in their granules that can readily degrade human fibronectin *in vitro* (DuBuske et al., J. Immunol. 1984, 133:1535-1541) into 8 or more fragments. Because this population of mast cells expresses mMCP-2 (Ghildyal et al., J. Biol. Chem. 1992, 267:8473-8477), mMCP-5 (McNeil et al., Proc. Natl. Acad. Sci. USA 1991, 89:11174-11178), mMCP-6 (Reynolds et al., J. Biol. Chem. 1991, 266:3847-3853), and mMCP-7 (McNeil et al., *supra*), it has not been ascertained which, if any, of these granule mMCPs degrade fibronectin *in vitro*. There are nearly 200 positively charged (Arg + Lys) residues in each subunit of fibronectin. Thus, it is not much of a surprise that this adhesion protein is susceptible to digestion by recombinant mMCP-6-FLAG. The novel finding is the specificity of the enzymatic attack when mMCP-6-FLAG is bound to heparin. Only 2 fragments are obtained after a 60-min incubation of fibronectin with mMCP-6-FLAG. N-terminal amino acid analysis of the amino acid sequence of the generated fragments is used to confirm that the preferred cleavage site in fibronectin is Arg-Gly-Arg-Gln-Lys-Thr (SEQ. ID NO. 11).

Swiss albino mouse skin-derived 3T3 fibroblasts exhibit homotypic, contact inhibition *in vitro*. However, these cells will become less adhesive and divide *in vitro* when they are trypsin treated. To determine if mMCP-6-FLAG could specifically alter the growth and/or adhesion of these cells, the fibroblasts were allowed to attach to replicate fibronectin-coated culture dishes and then were incubated for 15 min at 37°C with buffer alone or buffer containing either pro-EK-mMCP-6-FLAG, mMCP-6-FLAG, mMCP-7-FLAG, or trypsin. The fibroblasts which were exposed to buffer alone, pro-EK-mMCP-6-FLAG, or mMCP-7-FLAG continued to adhere to the fibronectin-coated culture dishes. Many of the cells in these cultures also exhibited the classical stellate shape of a fibroblast bound to its matrix via focal adhesion sites. In contrast,

both trypsin and mMCP-6-FLAG rapidly induced the cultured fibroblasts to round up. Moreover, very few fibroblasts remained attached to the culture dish after a 40 min incubation with either protease. SDS-PAGE/immunoblot analysis of the supernatants from the result cultures confirmed that fibronectin was degraded in the mMCP-6-FLAG-treated cultures but not in the pro-EK-mMCP-6-FLAG or mMCP-7-FLAG treated cultures.

Although Forsberg-Nilsson and coworkers (Scand. J. Immunol. 1996, 44:267-272) recently reported that a mast cell tryptase purified from human lung is not mitogenic for cultured human foreskin fibroblasts, Ruoss and coworkers (J. Clin. Invest. 1991, 88:493-499) reported that a tryptase purified from dog mastocytoma tissue is mitogenic for cultured Chinese hamster lung fibroblasts. Hartman and coworkers (Am. J. Physiol. 1992, 262:L528-L534) reported that a tryptase purified from human lung is mitogenic for cultured rat, hamster, and human fibroblasts but not for rat smooth muscle cells, and Cairns and Walls (J. Immunol. 1996, 156:275-283) reported that tryptases purified from human lung is mitogenic for the H292 human epithelial cell line. Mast cells express two or more tryptases in all species that have been examined. Moreover, strain-dependent expression of tryptase expression has been noted in mast cells of the mouse (Ghildyal et al., J. Immunol. 1994, 153:2624-2630; Hunt et al., J. Biol. Chem. 1996, 271:2851-2855) and rat (Lützelschwab et al., J. Exp. Med. 1996, 185:13-29). The discovery that mMCP-7-FLAG treated mouse fibroblasts do not lose their contact inhibition, continue to adhere to fibronectin, and do not increase their rate of proliferation, suggests that the apparently conflicting data in the above human studies probably is the result of functionally different tryptases in the analyzed preparations.

The mechanism by which the dog and human mast cell tryptases induce proliferation of fibroblasts and epithelial cells *in vitro* was not deduced in the Ruoss et al. (J. Clin. Invest. 1991, 88:493-499), Hartmann et al. (Am. J. Physiol. 1992, 262:L528-L534), and Cairns and Walls (J. Immunol. 1996, 156:275-283) studies but it appears that they do not stimulate cellular division via the thrombin receptor. While it is now well established that fibronectin plays a central role in cell adhesion, it has become increasingly apparent that certain proteolytically-derived fragments of fibronectin possess potent bioactivities in some *in vitro* systems. For example, the C-terminal 140- to 120-kDa fragment of fibronectin that presumably contains both its integrin- and syndecan-binding domains induces expression of certain metalloproteases and their inhibitors in fibroblasts and other cell types (Werb et al., J. Cell Biol. 1989, 109:877-889; Huhtala et al., J. Cell Biol. 1995, 129:867-879 ; Kapila et al., Matrix Biol. 1996, 15:251-261).

Relevant to our study, it has been shown that comparable fragments of fibronectin are chemotactic for fibroblasts (Seppä et al., Cell Biol. Int. Reports 1981, 5:813-819) and neutrophils (Odekon et al., Immunol. 1991, 74:114-120). The discovery that neutrophils are selectively recruited into the peritoneal cavity of BALB/c mice when recombinant mMCP-6-FLAG, but not recombinant mMCP-7-FLAG, is injected into this site, now suggests that the neutrophil emigration in this *in vivo* assay is mediated, in part, by a generated large-sized fragment of fibronectin that lacks its collagen binding domain. Thus, our discovery that the tryptase mMCP-6 (but not the tryptase mMCP-7) specifically cuts fibronectin between its collagen- and integrin-binding domains has important implications for mast cell-mediated control of fibrosis and inflammation.

Although mMCP-6 and mMCP-7 have different substrate specificities, both tryptases alter integrin-mediated signaling pathways. mMCP-7 does this by attacking fibrinogen which is the ligand for the $\alpha_M\beta_2$, $\alpha_X\beta_2$, $\alpha_{IIb}\beta_3$, and $\alpha_V\beta_3$, family of integrins (Springer Nature 1990, 346:425-434; Wahl et al., J. Leukocyte Biol. 1996, 59:789-796), whereas mMCP-6 does this by attacking fibronectin which is the ligand for the $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_V\beta_1$, $\alpha_{IIb}\beta_3$, $\alpha_V\beta_3$, and $\alpha_4\beta_7$ family of integrins (Springer, *supra* ; Wahl et al., *supra)* Although their roles in asthma have not been deduced, linkage analysis (De Sanctis et al., Nature Genetics 1995, 11:150-154) has implicated the region of chromosome 17 where the mMCP-6 and mMCP-7 genes reside as one of three candidate loci for the inheritance of intrinsic airway hyperresponsiveness. In addition, low molecular weight inhibitors of tryptic enzymes block antigen-induced airway constriction and tissue inflammatory response in *Ascaris suum*-sensitized sheep (Clark et al., Am. J. Respir. Crit. Care Med. 1995, 152:2076-2083). Our data suggest that mast cell tryptases play central roles in mast cell-mediated inflammation by controlling different integrin-dependent signaling pathways.

TABLE III presented below includes references to the GenBank Accession numbers of selected sequences presented in the Sequence Listing, followed by the claims and the abstract.

TABLE III.

SEQ ID NO:12	is the amino acid sequence of fibronectin (GenBank No. 279675)
SEQ ID NO:13	is the nucleotide sequence of mMCP-6 (GenBank No. M57625, Reynolds, et al., J. Biol. Chem. 1991, 266:3847-3853).
SEQ ID NO:14	is the nucleotide sequence of mMCP-6 (GenBank No. M57626, Reynolds, et al., J. Biol. Chem. 1991, 266:3847-3853).
SEQ ID NO:15	is the deduced amino acid sequence of the mMCP-6 zymogen (GenBank Nos. M57625 and M57626, Reynolds, et al., J. Biol. Chem. 1991, 266:3847-3853).
SEQ ID NO:16	is the nucleic acid sequence of human mast cell tryptase α (GenBank No. M30038).
SEQ ID NO:17	is the deduced amino acid sequence of human mast cell tryptase α (GenBank No. M30038).
SEQ ID NO:18	is the nucleic acid sequence of human mast cell tryptase I (GenBank No. M33491).
SEQ ID NO:19	is the deduced amino acid sequence of human mast cell tryptase I (GenBank No. M33491).
SEQ ID NO:20	is the nucleic acid sequence of human mast cell tryptase II/β (GenBank No. M33492).
SEQ ID NO:21	is the deduced amino acid sequence of human mast cell tryptase II/β (GenBank No. M33492).
SEQ ID NO:22	is the nucleic acid sequence of human mast cell tryptase III (GenBank No. M33493).
SEQ ID NO:23	is the deduced amino acid sequence of human mast cell tryptase III (GenBank No. M33493).
SEQ ID NO:24	is the nucleic acid sequence of the rat homolog of mMCP-6 (GenBank No. U67909)

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Brigham and Women's Hospital, Inc.

(ii) TITLE OF THE INVENTION: MAST CELL PROTEASE PEPTIDE INHIBITORS

(iii) NUMBER OF SEQUENCES: 65

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Wolf, Greenfield & Sacks, P.C.
(B) STREET: 600 Atlantic Avenue
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: U.S.A.
(F) ZIP: 02210-2211

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/037,090
(B) FILING DATE: 05-FEB-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Plumer, Elizabeth R.
(B) REGISTRATION NUMBER: 36,637
(C) REFERENCE/DOCKET NUMBER: B0801/7093

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617-720-3500
(B) TELEFAX: 617-720-2441
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Arg Asn Arg Gln Lys Thr

1 5

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Arg Asn Arg
1

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Arg Asn Arg Gln
1

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Asn Arg Gln Lys
1 5

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Asn Arg Gln Lys Thr
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Arg Gln Lys Thr
1

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gln Lys Thr
1

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Arg Gln Lys
1

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asn Arg Gln
1

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Gln Lys
1

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Arg Gly Arg Gln Lys Thr
 1               5
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2386 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Leu Arg Gly Pro Gly Pro Gly Leu Leu Leu Leu Ala Val Leu Cys
 1               5                  10                  15
Leu Gly Thr Ala Val Pro Ser Thr Gly Ala Ser Lys Ser Lys Arg Gln
            20                  25                  30
Ala Gln Gln Met Val Gln Pro Gln Ser Pro Val Ala Val Ser Gln Ser
        35                  40                  45
Lys Pro Gly Cys Tyr Asp Asn Gly Lys His Tyr Gln Ile Asn Gln Gln
    50                  55                  60
Trp Glu Arg Thr Tyr Leu Gly Asn Val Leu Val Cys Thr Cys Tyr Gly
65                  70                  75                  80
Gly Ser Arg Gly Phe Asn Cys Glu Ser Lys Pro Glu Ala Glu Glu Thr
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                85                  90                  95
Cys Phe Asp Lys Tyr Thr Gly Asn Thr Tyr Arg Val Gly Asp Thr Tyr
            100                 105                 110
Glu Arg Pro Lys Asp Ser Met Ile Trp Asp Cys Thr Cys Ile Gly Ala
        115                 120                 125
Gly Arg Gly Arg Ile Ser Cys Thr Ile Ala Asn Arg Cys His Glu Gly
    130                 135                 140
Gly Gln Ser Tyr Lys Ile Gly Asp Thr Trp Arg Arg Pro His Glu Thr
145                 150                 155                 160
Gly Gly Tyr Met Leu Glu Cys Val Cys Leu Gly Asn Gly Lys Gly Glu
                165                 170                 175
Trp Thr Cys Lys Pro Ile Ala Glu Lys Cys Phe Asp His Ala Ala Gly
            180                 185                 190
Thr Ser Tyr Val Val Gly Glu Thr Trp Glu Lys Pro Tyr Gln Gly Trp
        195                 200                 205
Met Met Val Asp Cys Thr Cys Leu Gly Glu Gly Ser Gly Arg Ile Thr
    210                 215                 220
Cys Thr Ser Arg Asn Arg Cys Asn Asp Gln Asp Thr Arg Thr Ser Tyr
225                 230                 235                 240
Arg Ile Gly Asp Thr Trp Ser Lys Lys Asp Asn Arg Gly Asn Leu Leu
                245                 250                 255
Gln Cys Ile Cys Thr Gly Asn Gly Arg Gly Glu Trp Lys Cys Glu Arg
            260                 265                 270
His Thr Ser Val Gln Thr Thr Ser Ser Gly Ser Gly Pro Phe Thr Asp
        275                 280                 285
Val Arg Ala Ala Val Tyr Gln Pro Gln Pro His Pro Gln Pro Pro Pro
    290                 295                 300
Tyr Gly His Cys Val Thr Asp Ser Gly Val Val Tyr Ser Val Gly Met
305                 310                 315                 320
Gln Trp Leu Lys Thr Gln Gly Asn Lys Gln Met Leu Cys Thr Cys Leu
                325                 330                 335
Gly Asn Gly Val Ser Cys Gln Glu Thr Ala Val Thr Gln Thr Tyr Gly
            340                 345                 350
Gly Asn Ser Asn Gly Glu Pro Cys Val Leu Pro Phe Thr Tyr Asn Gly
        355                 360                 365
Arg Thr Phe Tyr Ser Cys Thr Thr Glu Gly Arg Gln Asp Gly His Leu
    370                 375                 380
Trp Cys Ser Thr Thr Ser Asn Tyr Glu Gln Asp Gln Lys Tyr Ser Phe
385                 390                 395                 400
Cys Thr Asp His Thr Val Leu Val Gln Thr Gln Gly Gly Asn Ser Asn
                405                 410                 415
Gly Ala Leu Cys His Phe Pro Phe Leu Tyr Asn Asn His Asn Tyr Thr
            420                 425                 430
Asp Cys Thr Ser Glu Gly Arg Arg Asp Asn Met Lys Trp Cys Gly Thr
        435                 440                 445
Thr Gln Asn Tyr Asp Ala Asp Gln Lys Phe Gly Phe Cys Pro Met Ala
    450                 455                 460
Ala His Glu Glu Ile Cys Thr Thr Asn Glu Gly Val Met Tyr Arg Ile
465                 470                 475                 480
Gly Asp Gln Trp Asp Lys Gln His Asp Met Gly His Met Met Arg Cys
                485                 490                 495
Thr Cys Val Gly Asn Gly Arg Gly Glu Trp Thr Cys Tyr Ala Tyr Ser
            500                 505                 510
Gln Leu Arg Asp Gln Cys Ile Val Asp Asp Ile Thr Tyr Asn Val Asn
        515                 520                 525
Asp Thr Phe His Lys Arg His Glu Glu Gly His Met Leu Asn Cys Thr
    530                 535                 540
Cys Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys Asp Pro Val Asp Gln
```

545 550 555 560
Cys Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln Ile Gly Asp Ser Trp
565 570 575
Glu Lys Tyr Val His Gly Val Arg Tyr Gln Cys Tyr Cys Tyr Gly Arg
580 585 590
Gly Ile Gly Glu Trp His Cys Gln Pro Leu Gln Thr Tyr Pro Ser Ser
595 600 605
Ser Gly Pro Val Glu Val Phe Ile Thr Glu Thr Pro Ser Gln Pro Asn
610 615 620
Ser His Pro Ile Gln Trp Asn Ala Pro Gln Pro Ser His Ile Ser Lys
625 630 635 640
Tyr Ile Leu Arg Trp Arg Pro Lys Asn Ser Val Gly Arg Trp Lys Glu
645 650 655
Ala Thr Ile Pro Gly His Leu Asn Ser Tyr Thr Ile Lys Gly Leu Lys
660 665 670
Pro Gly Val Val Tyr Glu Gly Gln Leu Ile Ser Ile Gln Gln Tyr Gly
675 680 685
His Gln Glu Val Thr Arg Phe Asp Phe Thr Thr Thr Ser Thr Ser Thr
690 695 700
Pro Val Thr Ser Asn Thr Val Thr Gly Glu Thr Thr Pro Phe Ser Pro
705 710 715 720
Leu Val Ala Thr Ser Glu Ser Val Thr Glu Ile Thr Ala Ser Ser Phe
725 730 735
Val Val Ser Trp Val Ser Ala Ser Asp Thr Val Ser Gly Phe Arg Val
740 745 750
Glu Tyr Glu Leu Ser Glu Glu Gly Asp Glu Pro Gln Tyr Leu Asp Leu
755 760 765
Pro Ser Thr Ala Thr Ser Val Asn Ile Pro Asp Leu Leu Pro Gly Arg
770 775 780
Lys Tyr Ile Val Asn Val Tyr Gln Ile Ser Glu Asp Gly Glu Gln Ser
785 790 795 800
Leu Ile Leu Ser Thr Ser Gln Thr Thr Ala Pro Asp Ala Pro Pro Asp
805 810 815
Pro Thr Val Asp Gln Val Asp Asp Thr Ser Ile Val Val Arg Trp Ser
820 825 830
Arg Pro Gln Ala Pro Ile Thr Gly Tyr Arg Ile Val Tyr Ser Pro Ser
835 840 845
Val Glu Gly Ser Ser Thr Glu Leu Asn Leu Pro Glu Thr Ala Asn Ser
850 855 860
Val Thr Leu Ser Asp Leu Gln Pro Gly Val Gln Tyr Asn Ile Thr Ile
865 870 875 880
Tyr Ala Val Glu Glu Asn Gln Glu Ser Thr Pro Val Val Ile Gln Gln
885 890 895
Glu Thr Thr Gly Thr Pro Arg Ser Asp Thr Val Pro Ser Pro Arg Asp
900 905 910
Leu Gln Phe Val Glu Val Thr Asp Val Lys Val Thr Ile Met Trp Thr
915 920 925
Pro Pro Glu Ser Ala Val Thr Gly Tyr Arg Val Asp Val Ile Pro Val
930 935 940
Asn Leu Pro Gly Glu His Gly Gln Arg Leu Pro Ile Ser Arg Asn Thr
945 950 955 960
Phe Ala Glu Val Thr Gly Leu Ser Pro Gly Val Thr Tyr Tyr Phe Lys
965 970 975
Val Phe Ala Val Ser His Gly Arg Glu Ser Lys Pro Leu Thr Ala Gln
980 985 990
Gln Thr Thr Lys Leu Asp Ala Pro Thr Asn Leu Gln Phe Val Asn Glu
995 1000 1005
Thr Asp Ser Thr Val Leu Val Arg Trp Thr Pro Pro Arg Ala Gln Ile

1010 1015 1020
Thr Gly Tyr Arg Leu Thr Val Gly Leu Thr Arg Arg Gly Gln Pro Arg
025 1030 1035 1040
Gln Tyr Asn Val Gly Pro Ser Val Ser Lys Tyr Pro Leu Arg Asn Leu
1045 1050 1055
Gln Pro Ala Ser Glu Tyr Thr Val Ser Leu Val Ala Ile Lys Gly Asn
1060 1065 1070
Gln Glu Ser Pro Lys Ala Thr Gly Val Phe Thr Thr Leu Gln Pro Gly
1075 1080 1085
Ser Ser Ile Pro Pro Tyr Asn Thr Glu Val Thr Glu Thr Thr Ile Val
1090 1095 1100
Ile Thr Trp Thr Pro Ala Pro Arg Ile Gly Phe Lys Leu Gly Val Arg
105 1110 1115 1120
Pro Ser Gln Gly Gly Glu Ala Pro Arg Glu Val Thr Ser Asp Ser Gly
1125 1130 1135
Ser Ile Val Val Ser Gly Leu Thr Pro Gly Val Glu Tyr Val Tyr Thr
1140 1145 1150
Ile Gln Val Leu Arg Asp Gly Gln Glu Arg Asp Ala Pro Ile Val Asn
1155 1160 1165
Lys Val Val Thr Pro Leu Ser Pro Pro Thr Asn Leu His Leu Glu Ala
1170 1175 1180
Asn Pro Asp Thr Gly Val Leu Thr Val Ser Trp Glu Arg Ser Thr Thr
185 1190 1195 1200
Pro Asp Ile Thr Gly Tyr Arg Ile Thr Thr Thr Pro Thr Asn Gly Gln
1205 1210 1215
Gln Gly Asn Ser Leu Glu Glu Val Val His Ala Asp Gln Ser Ser Cys
1220 1225 1230
Thr Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr Asn Val Ser Val Tyr
1235 1240 1245
Thr Val Lys Asp Asp Lys Glu Ser Val Pro Ile Ser Asp Thr Ile Ile
1250 1255 1260
Pro Ala Val Pro Pro Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro
265 1270 1275 1280
Asp Thr Met Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr
1285 1290 1295
Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala
1300 1305 1310
Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu
1315 1320 1325
Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
1330 1335 1340
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp Ser
345 1350 1355 1360
Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe Thr Val
1365 1370 1375
His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg His
1380 1385 1390
His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp Arg Val Pro His
1395 1400 1405
Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr Pro Gly Thr Glu Tyr
1410 1415 1420
Val Val Ser Ile Val Ala Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu
425 1430 1435 1440
Ile Gly Gln Gln Ser Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val
1445 1450 1455
Val Ala Ala Thr Pro Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala
1460 1465 1470
Val Thr Val Arg Tyr Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn

1475 1480 1485
Ser Pro Val Gln Glu Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr
1490 1495 1500
Ile Ser Gly Leu Lys Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala
505 1510 1515 1520
Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile
1525 1530 1535
Asn Tyr Arg Thr Glu Ile Asp Lys Pro Ser Gln Met Gln Val Thr Asp
1540 1545 1550
Val Gln Asp Asn Ser Ile Ser Val Lys Trp Leu Pro Ser Ser Ser Pro
1555 1560 1565
Val Thr Gly Tyr Arg Val Thr Thr Thr Pro Lys Asn Gly Pro Gly Pro
1570 1575 1580
Thr Lys Thr Lys Thr Ala Gly Pro Asp Gln Thr Glu Met Thr Ile Glu
585 1590 1595 1600
Gly Leu Gln Pro Thr Val Glu Tyr Val Val Ser Val Tyr Ala Gln Asn
1605 1610 1615
Pro Ser Gly Glu Ser Gln Pro Leu Val Gln Thr Ala Val Thr Asn Ile
1620 1625 1630
Asp Arg Pro Lys Gly Leu Ala Phe Thr Asp Val Asp Val Asp Ser Ile
1635 1640 1645
Lys Ile Ala Trp Glu Ser Pro Gln Gly Gln Val Ser Arg Tyr Arg Val
1650 1655 1660
Thr Tyr Ser Ser Pro Glu Asp Gly Ile His Glu Leu Phe Pro Ala Pro
665 1670 1675 1680
Asp Gly Glu Glu Asp Thr Ala Glu Leu Gln Gly Leu Arg Pro Gly Ser
1685 1690 1695
Glu Tyr Thr Val Ser Val Val Ala Leu His Asp Asp Met Glu Ser Gln
1700 1705 1710
Pro Leu Ile Gly Thr Gln Ser Thr Ala Ile Pro Ala Pro Thr Asp Leu
1715 1720 1725
Lys Phe Thr Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro
1730 1735 1740
Pro Asn Val Gln Leu Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu
745 1750 1755 1760
Lys Thr Gly Pro Met Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser
1765 1770 1775
Val Val Val Ser Gly Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val
1780 1785 1790
Tyr Ala Leu Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val
1795 1800 1805
Thr Thr Leu Glu Asn Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp
1810 1815 1820
Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr
825 1830 1835 1840
Ile Thr Gly Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr Pro
1845 1850 1855
Ile Gln Arg Thr Ile Lys Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly
1860 1865 1870
Leu Gln Pro Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp
1875 1880 1885
Asn Ala Arg Ser Ser Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp
1890 1895 1900
Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu
905 1910 1915 1920
Val Ser Trp Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys
1925 1930 1935
Tyr Glu Lys Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg

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           1940                1945                1950
Pro Gly Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu
       1955                1960                1965
Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro
   1970                1975                1980
Leu Ile Gly Arg Lys Lys Thr Asp Glu Leu Pro Gln Leu Val Thr Leu
985                 1990                1995                2000
Pro His Pro Asn Leu His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
               2005                2010                2015
Val Gln Lys Thr Pro Phe Val Thr His Pro Gly Tyr Asp Thr Gly Asn
           2020                2025                2030
Gly Ile Gln Leu Pro Gly Thr Ser Gly Gln Gln Pro Ser Val Gly Gln
       2035                2040                2045
Gln Met Ile Phe Glu Glu His Gly Phe Arg Arg Thr Thr Pro Pro Thr
   2050                2055                2060
Thr Ala Thr Pro Ile Arg His Arg Pro Arg Pro Tyr Pro Pro Asn Val
065                 2070                2075                2080
Gly Glu Glu Ile Gln Ile Gly His Ile Pro Arg Glu Asp Val Asp Tyr
               2085                2090                2095
His Leu Tyr Pro His Gly Pro Gly Leu Asn Pro Asn Ala Ser Thr Gly
           2100                2105                2110
Gln Glu Ala Leu Ser Gln Thr Thr Ile Ser Trp Ala Pro Phe Gln Asp
       2115                2120                2125
Thr Ser Glu Tyr Ile Ile Ser Cys His Pro Val Gly Thr Asp Glu Glu
   2130                2135                2140
Pro Leu Gln Phe Arg Val Pro Gly Thr Ser Thr Ser Ala Thr Leu Thr
145                 2150                2155                2160
Gly Leu Thr Arg Gly Ala Thr Tyr Asn Ile Ile Val Glu Ala Leu Lys
               2165                2170                2175
Asp Gln Gln Arg His Lys Val Arg Glu Glu Val Val Thr Val Gly Asn
           2180                2185                2190
Ser Val Asn Glu Gly Leu Asn Gln Pro Thr Asp Asp Ser Cys Phe Asp
       2195                2200                2205
Pro Tyr Thr Val Ser His Tyr Ala Val Gly Asp Glu Trp Glu Arg Met
   2210                2215                2220
Ser Glu Ser Gly Phe Lys Leu Leu Cys Gln Cys Leu Gly Phe Gly Ser
225                 2230                2235                2240
Gly His Phe Arg Cys Asp Ser Ser Arg Trp Cys His Asp Asn Gly Val
               2245                2250                2255
Asn Tyr Lys Ile Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly Gln
           2260                2265                2270
Met Met Ser Cys Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys Cys
       2275                2280                2285
Asp Pro His Glu Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr His Val
   2290                2295                2300
Gly Glu Gln Trp Gln Lys Glu Tyr Leu Gly Ala Ile Cys Ser Cys Thr
305                 2310                2315                2320
Cys Phe Gly Gly Gln Arg Gly Trp Arg Cys Asp Asn Cys Arg Arg Pro
               2325                2330                2335
Gly Gly Glu Pro Ser Pro Glu Gly Thr Thr Gly Gln Ser Tyr Asn Gln
           2340                2345                2350
Tyr Ser Gln Arg Tyr His Gln Arg Thr Asn Thr Asn Val Asn Cys Pro
       2355                2360                2365
Ile Glu Cys Phe Met Pro Leu Asp Val Gln Ala Asp Arg Glu Asp Ser
   2370                2375                2380
Arg Glu
385
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3757 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GACGACCACT GCCAGGGACG AAAGTGCAAT GCGGCATACC TCAGTGGCGT GGAGTGCAGG     60
TATACAGATT AATCCGGCAG CGTCCGTCGT TGTTGATATT GCTTATGAAG GCTCCGGCAG    120
TGGCGACTGG CGTACTGACG GATTCATCGT TGGGGTCGGT TATAAATTCT GATTAGCCAG    180
GTAACACAGT GTTATGACAG CCCGCCGGAA CCGGTGGGCT TTTTTGTGGG GTGAATATGG    240
CAGTAAAGAT TTCAGGAGTC CTGAAAGACG GCACAGGAAA ACCGGTACAG AACTGCACCA    300
TTCAGCTGAA AGCCAGACGT AACAGCACCA CGGTGGTGGT GAACACGGTG GGCTCAGAGA    360
ATCCGGATGA AGCCTGCTTT TTTATACTAA GTTGGCATTA TAAAAAAGCA TTGCTTATCA    420
ATTTGTTGCA ACGAACAGGT CACTATCAGT CAAAATAAAA TCATTATTTG ATTTCAATTT    480
TGTCCCACTC CCTGCCTCTG TCATCACGAT ACTGTGATGC CATGGTGTCC GACTTATGCC    540
CGAGAAGATG TTGAGCAAAC TTATCGCTTA TCTGCTTCTC ATAGAGTCTT GCAGACAAAC    600
TGCGCAACTC GTGAAAGGTA GGCGGATCTG GGTCGACCTG CAGGTCAACG GATCCTCTCC    660
AGTGGAAAGC TGAGCCCAAC CCTGAGGACT CAGAGGATGC AAGATGAACG ACGCTGTTAC    720
CCATTGTGCT CTGCTCCTTG GGATGGCTCA CAGACACCAT CATCTCCTGT CCTGTCTCAC    780
TCTTGGGAAA TGTGTTAGAG TGTGTCAATA TGTCATGCTA GGGTGACACT GAGCCAGGAG    840
CCTTCTTGAG ACCTCTATAT CCCTGGGATG GGATCCCCAT CCCAATAGTT GGAAGGAGCA    900
GCGGCTCGGT GATGCAGAGC ACTCAACTGA GAGGCATCCT CAGTATGCGG TGCTCTGCCC    960
ACAGTGGACA GAGCAGACCT GGTGGAGGCA GAGCAGAGTA ACATCCTGAG CAGATGGGGG   1020
CCACGCCTGC CCAGGTCTCC TGATGTGGAG GGCTGCTTGT GGGACATCTG GCAAGCTCAG   1080
CATTTCCTTG GGCATTTCAC CGCTGAGGAA CAAGACATGA GGAGGAGGCA AATCTGAGAA   1140
GAGGCTACCA GCCTCCCCTC AGAAGATACC CCTTTCCAGG GAGGGCTGGG GATGACCACT   1200
GTCCTGCCAG CCCATCCACC CCACTACCTG ACTCTCCTAT CCTGGACCCA GAGCAGTTGC   1260
ATCTCTTAAC TCTGCCTTCC ATAGCCTGAA ATACCAAGAC TCTGTGTGTG TGTGTGTGTG   1320
TGTGTGTGTG TGTGTGTGTG TGTGTGTGTG TGTATGTGTA TGTGTGTGTG TATGACTGGT   1380
CCTCTCATTG TGCACTCAAC CGTGTGACCT GTGGTCATCA GAAGGGCATC TGGGTGGTGG   1440
GGACACATGT TACATGGAGG CCTTTGATCT AAATCACTAT TTCCTTTGTA TCTGGATTGG   1500
CGGGTGCTGT GTCCCTCCTC TCATGCACTC TGGTCTGGAG AATTAAAAAG GCAGAGGACA   1560
GCAGGCCAAG GAGAGAGGAG CAGAGACAGC TAAGGTAAAG TCCTGGTGTC TATATGTCAT   1620
CCTGAAGCAG AGTAACCAAG CTTGTGACCT TTGTAACCTG GTGCACCAAG CCCGCAGACT   1680
CCTGGGATGA ACCTGCCCTC CATCTCATGG GCCCTGGTTC CATTCTGGAC TTGATATTCT   1740
GCCAGCCCCA GTCCAGCCCT GTCTTCTAGC TGGACTCAGG CTGTGCTCCT CTCTGCTTCC   1800
AGATGCTGAA GCGGCGGCTG CTGCTGCTGT GGGCACTGTC CCTCCTGGCT AGTCTGGTGT   1860
ACTCAGCCCC TCGTAAGTTG TCTTGAGCCC TCCCTGTCTC TCCCTCACCT TCACAGGCCA   1920
CAGGAATGGG GAGTCTAGAG AATCCCAGGG TTAGCTCCAA TTCAGGAGGG GGCAAGGCAG   1980
GGCACAGAGG TTGCTTCTTG TCTCTCTCCA GGCCCAGCCA ATCAGCGAGT GGGCATCGTG   2040
GGAGGACATG AGGCTTCTGA GAGTAAGTGG CCCTGGCAGG TGAGCCTGAG ATTTAAATTA   2100
AACTACTGGA TACATTTCTG CGGAGGCTCT CTCATCCACC CACAGTGGGT GCTCACTGCG   2160
GCACACTGTG TGGGACCGTG AGTCTCCCTG GGCCTGGCAT GGTGGGACGG GATCTAGATT   2220
ATTCCCACCA TCCCCAGTGT TCCCGAGGAT GTGCCCATCC TGGCTGGAGC CTTCTGAGCA   2280
TGATTATACT CTTCTAGGCA CATCAAAAGC CCACAGCTCT TCCGGGTGCA GCTTCGTGAG   2340
CAGTATCTAT ACTATGGGGA CCAGCTCCTC TCTTTGAACC GGATCGTGGT GCACCCCCAC   2400
TATTACACGG CCGAGGGTGG GGCAGACGTT GCCCTGCTGG AGCTTGAGGT CCCTGTGAAT   2460
GTCTCCACCC ATATCCACCC CATATCCCTG CCCCCTGCCT CGGAGACCTT CCCCCCTGGG   2520
ACATCGTGCT GGGTGACAGG CTGGGGCGAC ATTGATAATG ACGGTATGTG GCAAGGATAG   2580
CTGACAGTTA GGCAGGGACT AAGTCTCCTC CAATCCCAGC ATTGGAGGGT GGGCAGGGAT   2640
TCCAGTGGCT GGTTACTCTT GAGCCTCCCT CAAAGGCTGC ACTTGTCCCA CCCCAGAGCC   2700
TCTCCCACCT CCTTATCCTC TGAAGCAAGT GAAGGTTCCC ATTGTGGAAA ACAGCCTGTG   2760
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TGACCGGAAG TACCACACTG GCCTCTACAC GGGAGATGAT TTTCCCATTG TCCATGATGG 2820
CATGCTGTGT GCTGGAAATA CCAGGAGAGA CTCCTGCCAG GTAGGTCCTG TGTCCTCCCT 2880
GCACCACACC CCATCTGGTC TCCATACTGT GTGCTGACCC CTGTCTTCTT CAGGGCGATT 2940
CAGGGGGGCC ACTGGTCTGC AAAGTGAAGG GTACCTGGCT GCAGGCAGGA GTGGTCAGCT 3000
GGGGTGAGGG CTGCGCACAG CCCAACAAGC CTGGCATCTA CACCCGGGTG ACATACTACT 3060
TAGACTGGAT CCACCGCTAT GTCCCTGAGC ATTCCTGAGA CCTATCCAGG GTCAGGCAAG 3120
AACCAGGGCC GTGCTGTCTT TAACTCACTG CTTCCTGGTC AGGTGGAACC CTTGCCTTCC 3180
TTGTCCTCTG TCTCCCCTGT CTACTAGGTG TCCCTCTGAG GCCCCCACCC CCCAGTTCCG 3240
TCTTGAGTCC CTAGCCATTC CGGTTCCCTC TTGCCTCCCA CCACATAATA GTTGCATTGT 3300
GTGGCTCCCT CTCTTCTGTG GCTCATTAAA GTACTTGAAA ACAGCTATTG GAGTTGCTTC 3360
AAGAGTTCAA GGTCATCCTT GTCTATGTAT TGAGGTCGAG GCCAGTCTGG GATATGTGAG 3420
GCACCATCCC AAGACCATAA AGATCAAAAA TAAGTTCATG CAGCGGCACA TTTGCCTGCT 3480
ACAGTACACA ACATCACATC TGGCTGCTCC AGTCATGCAG TGGTACATCT GGCTGCTCCA 3540
GTCACATAGG AGCACATCTG GCTGCTCCAG TCATGCAGTG GTACATCTGG CTGCTCCAGT 3600
CACATAGGAG CACATCTGGC TGCTCCAGTC ACTTTGCTTT GGGTATTCTC ATTTGAGCCT 3660
CTTGGCCCTT GGGTGCTCAT GGCCATTCCT GCACACACAC ATATGCTTAT ATCTGGAACT 3720
TTCTGCTGAA GGGAGCTGTT GGTTCATGAA TAGGCCC 3757

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATCCAATTGA AGAGAGGAGC AGAGACAGCT AAGATGCTGA AGCGGCGGCT GCTGCTGCTG 60
TGGGCACTGT CCCTCCTGGC TAGTCTGGTG TACTCAGCCC CTCGCCCAGC CAATCAGCGA 120
GTGGGCATCG TGGGAGGACA TGAGGCTTCT GAGAGTAAGT GGCCCTGGCA GGTGAGCCTG 180
AGATTTAAAT TAAACTACTG GATACATTTC TGCGGAGGCT CTCTCATCCA CCCACAGTGG 240
GTGCTCACTG CGGCACACTG TGTGGGACCG CACATCAAAA GCCCACAGCT CTTCCGGGTG 300
CAGCTTCGTG AGCAGTATCT ATACTATGGG GACCAGCTCC TCTCTTTGAA CCGGATCGTG 360
GTGCACCCCC ACTATTACAC GGCCGAGGGT GGGGCAGACG TTGCCCTGCT GGAGCTTGAG 420
GTCCCTGTGA ATGTCTCCAC CCATATCCAC CCCATATCCC TGCCCCCTGC CTCGGAGACC 480
TTCCCCCCTG GGACATCGTG CTGGGTGACA GGCTGGGGCG ACATTGATAA TGACGAGCCT 540
CTCCCACCTC CTTATCCTCT GAAGCAAGTG AAGGTTCCCA TTGTGGAAAA CAGCCTGTGT 600
GACCGGAAGT ACCACACTGG CCTCTACACG GGAGATGATT TTCCCATTGT CCATGATGGC 660
ATGCTGTGTG CTGGAAATAC CAGGAGAGAC TCCTGCCAGG GCGATTCAGG GGGGCCACTG 720
GTCTGCAAAG TGAAGGGTAC CTGGCTGCAG GCAGGAGTGG TCAGCTGGGG TGAGGGCTGC 780
GCACAGCCCA ACAAGCCTGG CATCTACACC CGGGTGACAT ACTACTTAGA CTGGATCCAC 840
CGCTATGTCC CTGAGCATTC CTGAGACCTA TCCAGGGTCA GGCAAGAACC AGGGCCGTGC 900
TGTCTTTAAC TCACTGCTTC CTGGTCAGGT GGAACCCTTG CCTTCCTTGT CCTCTGTCTC 960
CCCTGTCTAC TAGGTGTCCC TCTGAGGCCC CCACCCCCCA GTTCCGTCTT GAGTCCCTAG 1020
CCATTCCGGT TCCCTCTTGC CTCCCACCAC ATAATAGTTG CATTGTGTGG CTCCCTCTCT 1080
TCTGTGGCTC ATTAAAGTAC TTGAAAAC 1108

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 276 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Met Leu Lys Arg Arg Leu Leu Leu Leu Trp Ala Leu Ser Leu Leu Ala
 1               5                  10                  15
Ser Leu Val Tyr Ser Ala Pro Arg Pro Ala Asn Gln Arg Val Gly Ile
            20                  25                  30
Val Gly Gly His Glu Ala Ser Glu Ser Lys Trp Pro Trp Gln Val Ser
        35                  40                  45
Leu Arg Phe Lys Leu Asn Tyr Trp Ile His Phe Cys Gly Gly Ser Leu
    50                  55                  60
Ile His Pro Gln Trp Val Leu Thr Ala Ala His Cys Val Gly Pro His
65                  70                  75                  80
Ile Lys Ser Pro Gln Leu Phe Arg Val Gln Leu Arg Glu Gln Tyr Leu
                85                  90                  95
Tyr Tyr Gly Asp Gln Leu Leu Ser Leu Asn Arg Ile Val Val His Pro
            100                 105                 110
His Tyr Tyr Thr Ala Glu Gly Gly Ala Asp Val Ala Leu Leu Glu Leu
        115                 120                 125
Glu Val Pro Val Asn Val Ser Thr His Ile His Pro Ile Ser Leu Pro
    130                 135                 140
Pro Ala Ser Glu Thr Phe Pro Pro Gly Thr Ser Cys Trp Val Thr Gly
145                 150                 155                 160
Trp Gly Asp Ile Asp Asn Asp Glu Pro Leu Pro Pro Pro Tyr Pro Leu
                165                 170                 175
Lys Gln Val Lys Val Pro Ile Val Glu Asn Ser Leu Cys Asp Arg Lys
            180                 185                 190
Tyr His Thr Gly Leu Tyr Thr Gly Asp Asp Phe Pro Ile Val His Asp
        195                 200                 205
Gly Met Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly Asp
    210                 215                 220
Ser Gly Gly Pro Leu Val Cys Lys Val Lys Gly Thr Trp Leu Gln Ala
225                 230                 235                 240
Gly Val Val Ser Trp Gly Glu Gly Cys Ala Gln Pro Asn Lys Pro Gly
                245                 250                 255
Ile Tyr Thr Arg Val Thr Tyr Tyr Leu Asp Trp Ile His Arg Tyr Val
            260                 265                 270
Pro Glu His Ser
        275
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1154 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
GGAATTCCGT GGCCAGGATG CTGAGCCTGC TGCTGCTGGC GCTGCCCGTC CTGGCGAGCC     60
GCGCCTACGC GGCCCCTGCC CCAGTCCAGG CCCTGCAGCA AGCGGGTATC GTCGGGGGTC    120
AGGAGGCCCC CAGGAGCAAG TGGCCCTGGC AGGTGAGCCT GAGAGTCCGC GACCGATACT    180
GGATGCACTT CTGCGGGGGC TCCCTCATCC ACCCCCAGTG GGTGCTGACC GCGGCGCACT    240
GCCTGGGACC GGACGTCAAG GATCTGGCCA CCCTCAGGGT GCAACTGCGG GAGCAGCACC    300
```

```
TCTACTACCA GGACCAGCTG CTGCCAGTCA GCAGGATCAT CGTGCACCCA CAGTTCTACA    360
TCATCCAGAC TGGAGCGGAT ATCGCCCTGC TGGAGCTGGA GGAGCCCGTG AACATCTCCA    420
GCCGCGTCCA CACGGTCATG CTGCCCCCTG CCTCGGAGAC CTTCCCCCCG GGGATGCCGT    480
GCTGGGTCAC TGGCTGGGGC GATGTGGACA ATGATGAGCC CCTCCCACCG CCATTTCCCC    540
TGAAGCAGGT GAAGGTCCCC ATAATGGAAA ACCACATTTG TGACGCAAAA TACCACCTTG    600
GCGCCTACAC GGGAGACGAC GTCCGCATCA TCCGTGACGA CATGCTGTGT GCCGGGAACA    660
GCCAGAGGGA CTCCTGCAAG GGCGACTCTG GAGGGCCCCT GGTGTGCAAG GTGAATGGCA    720
CCTGGCTACA GGCGGGCGTG GTCAGCTGGG ACGAGGGCTG TGCCCAGCCC AACCGGCCTG    780
GCATCTACAC CCGTGTCACC TACTACTTGG ACTGGATCCA CCACTATGTC CCCAAAAAGC    840
CGTGAGTCAG GCCTGGGTGT GCCACCTGGG TCACTGGAGG ACCAACCCCT GCTGTCCAAA    900
ACACCACTGC TTCCTACCCA GGTGGCGACT GCCCCCCACA CCTTCCCTGC CCCGTCCTGA    960
GTGCCCCTTC CTGTCCTAAG CCCCCTGCTC TCTTCTGAGC CCCTTCCCCT GTCCTGAGGA   1020
CCCTTCCCCA TCCTGAGCCC CCTTCCCTGT CCTAAGCCTG ACGCCTGCAC TGCTCCGGCC   1080
CTCCCCTGCC CAGGCAGCTG GTGGTGGGCG CTAATCCTCC TGAGTGCTGG ACCTCATTAA   1140
AGTGCATGGA AATC                                                     1154
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 275 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Met Leu Ser Leu Leu Leu Leu Ala Leu Pro Val Leu Ala Ser Arg Ala
 1               5                  10                  15
Tyr Ala Ala Pro Ala Pro Val Gln Ala Leu Gln Gln Ala Gly Ile Val
            20                  25                  30
Gly Gly Gln Glu Ala Pro Arg Ser Lys Trp Pro Trp Gln Val Ser Leu
        35                  40                  45
Arg Val Arg Asp Arg Tyr Trp Met His Phe Cys Gly Gly Ser Leu Ile
    50                  55                  60
His Pro Gln Trp Val Leu Thr Ala Ala His Cys Leu Gly Pro Asp Val
65                  70                  75                  80
Lys Asp Leu Ala Thr Leu Arg Val Gln Leu Arg Glu Gln His Leu Tyr
                85                  90                  95
Tyr Gln Asp Gln Leu Leu Pro Val Ser Arg Ile Ile Val His Pro Gln
            100                 105                 110
Phe Tyr Ile Ile Gln Thr Gly Ala Asp Ile Ala Leu Leu Glu Leu Glu
        115                 120                 125
Glu Pro Val Asn Ile Ser Ser Arg Val His Thr Val Met Leu Pro Pro
    130                 135                 140
Ala Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr Gly Trp
145                 150                 155                 160
Gly Asp Val Asp Asn Asp Glu Pro Leu Pro Pro Pro Phe Pro Leu Lys
                165                 170                 175
Gln Val Lys Val Pro Ile Met Glu Asn His Ile Cys Asp Ala Lys Tyr
            180                 185                 190
His Leu Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Ile Arg Asp Asp
        195                 200                 205
Met Leu Cys Ala Gly Asn Ser Gln Arg Asp Ser Cys Lys Gly Asp Ser
    210                 215                 220
Gly Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln Ala Gly
225                 230                 235                 240
```

```
Val Val Ser Trp Asp Glu Gly Cys Ala Gln Pro Asn Arg Pro Gly Ile
                245                 250                 255
Tyr Thr Arg Val Thr Tyr Tyr Leu Asp Trp Ile His His Tyr Val Pro
            260                 265                 270
Lys Lys Pro
        275
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1137 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
TGAATCTGCT GCTGCTGGCG CTGCCCGTCC TGGCGAGCCG CGCCTACGCG GCCCCTGCCC     60
CAGGCCAGGC CCTGCAGCGA GTGGGCATCG TCGGGGGTCA GGAGGCCCCC AGGAGCAAGT    120
GGCCCTGGCA GGTGAGCCTG AGAGTCCACG GCCCATACTG GATGCACTTC TGCGGGGGCT    180
CCCTCATCCA CCCCCAGTGG GTGCTGACCG CAGCGCACTG CGTGGGACCG GACGTCAAGG    240
ATCTGGCCGC CCTCAGGGTG CAACTGCGGG AGCAGCACCT CTACTACCAG GACCAGCTGC    300
TGCCGGTCAG CAGGATCATC GTGCACCCAC AGTTCTACAC CGCCCAGATC GGAGCGGACA    360
TCGCCCTGCT GGAGCTGGAG GAGCCGGTGA ACGTCTCCAG CCACGTCCAC ACGGTCACCC    420
TGCCCCCTGC CTCAGAGACC TTCCCCCCGG GGATGCCGTG CTGGGTCACT GGCTGGGGCG    480
ATGTGGACAA TGATGAGCGC CTCCCACCGC CATTTCCTCT GAAGCAGGTG AAGGTCCCCA    540
TAATGGAAAA CCACATTTGT GACGCAAAAT ACCACCTTGG CGCCTACACG GGAGACGACG    600
TCCGCATCGT CCGTGACGAC ATGCTGTGTG CCGGGAACAC CCGGAGGGAC TCATGCCAGG    660
GCGACTCCGG AGGGCCCCTG GTGTGCAAGG TGAATGGCAC CTGGCTGCAG GCGGGCGTGG    720
TCAGCTGGGG CGAGGGCTGT GCCCAGCCCA ACCGGCCTGG CATCTACACC CGTGTCACCT    780
ACTACTTGGA CTGGATCCAC CACTATGTCC CCAAAAAGCC GTGAGTCAGG CCTGGGTTGG    840
CCACCTGGGT CACTGGAGGA CCAACCCCTG CTGTCCAAAA CACCACTGCT TCCTACCCAG    900
GTGGCGACTG CCCCCCACAC CTTCCCTGCC CCGTCCTGAG TGCCCCTTCC TGTCCTAAGC    960
CCCCTGCTCT CTTCTGAGCC CCTTCCCCTG TCCTGAGGAC CCTTCCCTAT CCTGAGCCCC   1020
CTTCCCTGTC CTAAGCCTGA CGCCTGCACC GGGCCCTCCA GCCCTCCCCT GCCCAGATAG   1080
CTGGTGGTGG GCGCTAATCC TCCTGAGTGC TGGACCTCAT TAAAGTGCAT GGAAATC      1137
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 273 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Asn Leu Leu Leu Leu Ala Leu Pro Val Leu Ala Ser Arg Ala Tyr Ala
 1               5                  10                  15
Ala Pro Ala Pro Gly Gln Ala Leu Gln Arg Val Gly Ile Val Gly Gly
            20                  25                  30
Gln Glu Ala Pro Arg Ser Lys Trp Pro Trp Gln Val Ser Leu Arg Val
        35                  40                  45
His Gly Pro Tyr Trp Met His Phe Cys Gly Gly Ser Leu Ile His Pro
    50                  55                  60
```

```
Gln Trp Val Leu Thr Ala Ala His Cys Val Gly Pro Asp Val Lys Asp
65                  70                  75                  80
Leu Ala Ala Leu Arg Val Gln Leu Arg Glu Gln His Leu Tyr Tyr Gln
                85                  90                  95
Asp Gln Leu Leu Pro Val Ser Arg Ile Ile Val His Pro Gln Phe Tyr
            100                 105                 110
Thr Ala Gln Ile Gly Ala Asp Ile Ala Leu Leu Glu Leu Glu Glu Pro
        115                 120                 125
Val Asn Val Ser Ser His Val His Thr Val Thr Leu Pro Pro Ala Ser
    130                 135                 140
Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr Gly Trp Gly Asp
145                 150                 155                 160
Val Asp Asn Asp Glu Arg Leu Pro Pro Pro Phe Pro Leu Lys Gln Val
                165                 170                 175
Lys Val Pro Ile Met Glu Asn His Ile Cys Asp Ala Lys Tyr His Leu
            180                 185                 190
Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Val Arg Asp Asp Met Leu
        195                 200                 205
Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly Asp Ser Gly Gly
    210                 215                 220
Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln Ala Gly Val Val
225                 230                 235                 240
Ser Trp Gly Glu Gly Cys Ala Gln Pro Asn Arg Pro Gly Ile Tyr Thr
                245                 250                 255
Arg Val Thr Tyr Tyr Leu Asp Trp Ile His His Tyr Val Pro Lys Lys
            260                 265                 270
Pro
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1128 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
GCTGAATCTG CTGCTGCTGG CGCTGCCCGT CCTGGCGAGC CGCGCCTACG CGGCCCCTGC     60
CCCAGGCCAG GCCCTGCAGC GAGTGGGCAT CGTTGGGGGT CAGGAGGCCC CCAGGAGCAA    120
GTGGCCCTGG CAGGTGAGCC TGAGAGTCCA CGGCCCATAC TGGATGCACT TCTGCGGGGG    180
CTCCCTCATC CACCCCCAGT GGGTGCTGAC CGCAGCGCAC TGCGTGGGAC CGGACGTCAA    240
GGATCTGGCC GCCCTCAGGG TGCAACTGCG GGAGCAGCAC CTCTACTACC AGGACCAGCT    300
GCTGCCGGTC AGCAGGATCA TCGTGCACCC ACAGTTCTAC ACCGCCCAGA TCGGAGCGGA    360
CATCGCCCTG CTGGAGCTGG AGGAGCCGGT GAAGGTCTCC AGCCACGTCC ACACGGTCAC    420
CCTGCCCCCT GCCTCAGAGA CCTTCCCCCC GGGGATGCCG TGCTGGGTCA CTGGCTGGGG    480
CGATGTGGAC AATGATGAGC GCCTCCCACC GCCATTTCCT CTGAAGCAGG TGAAGGTCCC    540
CATAATGGAA AACCACATTT GTGACGCAAA ATACCACCTT GGCGCCTACA CGGGAGACGA    600
CGTCCGCATC GTCCGTGACG ACATGCTGTG TGCCGGGAAC ACCCGGAGGG ACTCATGCCA    660
GGGCGACTCC GGAGGGCCCC TGGTGTGCAA GGTGAATGGC ACCTGGCTGC AGGCGGGCGT    720
GGTCAGCTGG GGCGAGGGCT GTGCCCAGCC CAACCGGCCT GGCATCTACA CCCGTGTCAC    780
CTACTACTTG GACTGGATCC ACCACTATGT CCCCAAAAAG CCGTGAGTCA GGCCTGGGTT    840
GGCCACCTGG GTCACTGGAG GACCAACCCC TGCTGTCCAA AACACCACTG CTTCCTACCC    900
AGGTGGCGAC TGCCCCCCAC ACCTTCCCTG CCCCGTCCTG AGTGCCCCTT CCTGTCCTAA    960
GCCCCCTGCT CTCTTCTGAG CCCCTTCCCC TGTCCTGAGG ACCCTTCCCC ATCCTGAGCC   1020
```

CCCTTCCCTG TCCTAAGCCT GACGCCTGCA CCGGGCCCTC CGGCCCTCCC CTGCCCAGGC 1080
AGCTGGTGGT GGGCGCTAAT CCTCCTGAGT GCTGGACCTC ATTAAAGT 1128

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 274 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Leu Asn Leu Leu Leu Leu Ala Leu Pro Val Leu Ala Ser Arg Ala Tyr
 1               5                  10                  15
Ala Ala Pro Ala Pro Gly Gln Ala Leu Gln Arg Val Gly Ile Val Gly
            20                  25                  30
Gly Gln Glu Ala Pro Arg Ser Lys Trp Pro Trp Gln Val Ser Leu Arg
        35                  40                  45
Val His Gly Pro Tyr Trp Met His Phe Cys Gly Gly Ser Leu Ile His
    50                  55                  60
Pro Gln Trp Val Leu Thr Ala Ala His Cys Val Gly Pro Asp Val Lys
65                  70                  75                  80
Asp Leu Ala Ala Leu Arg Val Gln Leu Arg Glu Gln His Leu Tyr Tyr
                85                  90                  95
Gln Asp Gln Leu Leu Pro Val Ser Arg Ile Ile Val His Pro Gln Phe
            100                 105                 110
Tyr Thr Ala Gln Ile Gly Ala Asp Ile Ala Leu Leu Glu Leu Glu Glu
        115                 120                 125
Pro Val Lys Val Ser Ser His Val His Thr Val Thr Leu Pro Pro Ala
    130                 135                 140
Ser Glu Thr Phe Pro Pro Gly Met Pro Cys Trp Val Thr Gly Trp Gly
145                 150                 155                 160
Asp Val Asp Asn Asp Glu Arg Leu Pro Pro Pro Phe Pro Leu Lys Gln
                165                 170                 175
Val Lys Val Pro Ile Met Glu Asn His Ile Cys Asp Ala Lys Tyr His
            180                 185                 190
Leu Gly Ala Tyr Thr Gly Asp Asp Val Arg Ile Val Arg Asp Asp Met
        195                 200                 205
Leu Cys Ala Gly Asn Thr Arg Arg Asp Ser Cys Gln Gly Asp Ser Gly
    210                 215                 220
Gly Pro Leu Val Cys Lys Val Asn Gly Thr Trp Leu Gln Ala Gly Val
225                 230                 235                 240
Val Ser Trp Gly Glu Gly Cys Ala Gln Pro Asn Arg Pro Gly Ile Tyr
                245                 250                 255
Thr Arg Val Thr Tyr Tyr Leu Asp Trp Ile His His Tyr Val Pro Lys
            260                 265                 270
Lys Pro
```

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1081 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
GCTGCCCGTC CTGGCGAGCC GCGCCTACGC GGCCCCTGCC CCAGGCCAGG CCCTGCAGCG     60
AGTGGGCATC GTTGGGGGTC AGGAGGCCCC CAGGAGCAAG TGGCCCTGGC AGGTGAGCCT    120
GAGAGTCCGC GACCGATACT GGATGCACTT CTGCGGGGGC TCCCTCATCC ACCCCCAGTG    180
GGTGCTGACC GCAGCGCACT GCGTGGGACC GGACGTCAAG GATCTGGCCG CCCTCAGGGT    240
GCAACTGCGG GAGCAGCACC TCTACTACCA GGACCAGCTG CTGCCGGTCA GCAGGATCAT    300
CGTGCACCCA CAGTTCTACA CCGCCCAGAT CGGAGCGGAC ATCGCCCTGC TGGAGCTGGA    360
GGAGCCGGTG AAGGTCTCCA GCCACGTCCA CACGGTCACC CTGCCCCCTG CCTCAGAGAC    420
CTTCCCCCCG GGGATGCCGT GCTGGGTCAC TGGCTGGGGC GATGTGGACA ATGATGAGCG    480
CCTCCCACCG CCATTTCCTC TGAAGCAGGT GAAGGTCCCC ATAATGGAAA ACCACATTTG    540
TGACGCAAAA TACCACCTTG GCGCCTACAC GGGAGACGAC GTCCGCATCG TCCGTGACGA    600
CATGCTGTGT GCCGGGAACA CCCGGAGGGA CTCATGCCAG GGCGACTCCG GAGGGCCCCT    660
GGTGTGCAAG GTGAATGGCA CCTGGCTGCA GGCGGGCGTG GTCAGCTGGG GCGAGGGCTG    720
TGCCCAGCCC AACCGGCCTG GCATCTACAC CCGTGTCACC TACTACTTGG ACTGGATCCA    780
CCACTATGTC CCCAAAAAGC CGTGAGTCAG GCCTGGGGTG TCCACCTGGG TCACTGGAGG    840
ACCAGCCCCT CCTGTCCAAA ACACCACTGC TTCCTACCCA GGCGGCGACT GCCCCCCACA    900
CCTTCCCTGC CCCGTCCTGA GTGCCCCTTC CTGTCCTAAG CCCCCTGCTC TCTTCTGAGC    960
CCCTTCCCCT GTCCTGAGGA CCCTTCCCCA TCCTGAGCCC CCTTCCCTGT CCTAAGCCTG   1020
ACGCCTGCAC CGGGCCCTCC GGCCCTCCCC TGCCCAGGCA GCTGGTGGTG GGCGCTAATC   1080
C                                                                   1081
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 267 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Leu Pro Val Leu Ala Ser Arg Ala Tyr Ala Ala Pro Ala Pro Gly Gln
 1               5                  10                  15
Ala Leu Gln Arg Val Gly Ile Val Gly Gly Gln Glu Ala Pro Arg Ser
            20                  25                  30
Lys Trp Pro Trp Gln Val Ser Leu Arg Val Arg Asp Arg Tyr Trp Met
        35                  40                  45
His Phe Cys Gly Gly Ser Leu Ile His Pro Gln Trp Val Leu Thr Ala
    50                  55                  60
Ala His Cys Val Gly Pro Asp Val Lys Asp Leu Ala Ala Leu Arg Val
65                  70                  75                  80
Gln Leu Arg Glu Gln His Leu Tyr Tyr Gln Asp Gln Leu Leu Pro Val
                85                  90                  95
Ser Arg Ile Ile Val His Pro Gln Phe Tyr Thr Ala Gln Ile Gly Ala
            100                 105                 110
Asp Ile Ala Leu Leu Glu Leu Glu Glu Pro Val Lys Val Ser Ser His
        115                 120                 125
Val His Thr Val Thr Leu Pro Pro Ala Ser Glu Thr Phe Pro Pro Gly
    130                 135                 140
Met Pro Cys Trp Val Thr Gly Trp Gly Asp Val Asp Asn Asp Glu Arg
145                 150                 155                 160
Leu Pro Pro Pro Phe Pro Leu Lys Gln Val Lys Val Pro Ile Met Glu
                165                 170                 175
Asn His Ile Cys Asp Ala Lys Tyr His Leu Gly Ala Tyr Thr Gly Asp
```

```
            180                 185                 190
Asp Val Arg Ile Val Arg Asp Asp Met Leu Cys Ala Gly Asn Thr Arg
        195                 200                 205
Arg Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Lys Val
    210                 215                 220
Asn Gly Thr Trp Leu Gln Ala Gly Val Val Ser Trp Gly Glu Gly Cys
225                 230                 235                 240
Ala Gln Pro Asn Arg Pro Gly Ile Tyr Thr Arg Val Thr Tyr Tyr Leu
                245                 250                 255
Asp Trp Ile His His Tyr Val Pro Lys Lys Pro
            260                 265
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1103 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
TGCCGAGACA GCCAAGATGC TGAAGCTGCT GCTGCTGCTG GCACTGTCCC CCCTGGCTAG     60
TCTGGTGCAC GCGGCCCCTT GCCCAGTCAA GCAGCGAGTG GCATTGTGG GAGGACGAGA    120
GGCTTCTGAA AGTAAGTGGC CCTGGCAGGT GAGCCTGAGA TTTAAATTCA GCTTCTGGAT    180
GCATTTCTGT GGCGGCTCCC TCATTCACCC ACAGTGGGTG CTCACTGCGG CACACTGTGT    240
GGGACTGCAC ATCAAAAGCC CAGAGCTCTT CCGTGTACAG CTTCGTGAGC AGTATCTATA    300
CTATGCGGAC CAGCTACTGA CTGTGAACCG GACCGTTGTG CACCCCCACT ACTACACAGT    360
CGAGGATGGG GCAGACATTG CCCTGCTGGA GCTTGAGAAC CCTGTGAATG TCTCCACCCA    420
TATCCACCCC ACATCCCTGC CCCCTGCCTC GGAGACCTTC CCCTCGGGGA CTTCTTGCTG    480
GGTAACAGGC TGGGGCGACA TTGATAGTGA CGAGCCTCTC CTGCCACCTT ATCCTCTGAA    540
GCAAGTGAAG GTCCCCATTG TGGAAAACAG CCTGTGTGAT CGGAAGTACC ACACTGGCCT    600
CTACACAGGA GATGATGTTC CCATTGTCCA GGATGGCATG CTGTGTGCTG GAAATACCAG    660
GAGCGACTCC TGCCAGGGAG ACTCAGGGGG CCCACTGGTC TGCAAAGTGA AGGGTACCTG    720
GCTGCAAGCA GGAGTGGTCA GCTGGGGCGA GGGCTGCGCA GAGGCCAATC GTCCTGGCAT    780
TTACACCCGG GTGACGTACT ACCTGGACTG GATTCACCGC TATGTCCCTC AGCGTTCCTG    840
AGACCCATCC AGGGTCAGGG AAGAACCAGG CACCTGCTGT CTTTAACTCA CTGCTTCCTG    900
GCCAGATGGA ACCCTGGCCT TCTTTGTACT CTGTCTCCCC TGTCTTCCGG GTGTCCCTCT    960
GAGCCCCCAC TTTGTTCCAC CTTGAGTCCC TCGCCACTCC TGTCCCCTCT GCCTCCCACC   1020
ACACACAGCT GCACTGTGCG GCTCCCTCTT TTCTGTGGCT CATTAAAGTA TGTGAAAATT   1080
TTGCTCCAAA AAAAAAAAAA AAA                                           1103
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Ala Pro Gly Pro Ala Met Thr Arg Glu Gly
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ala Pro Arg Pro Ala Asn Gln Arg Val Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ala Pro Val Gln Ala Leu Gln Gln Ala Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Pro Gly Gln Ala Leu Gln Arg Val Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Asp Asp Asp Asp Lys

1 5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Val Arg Pro Val Lys Ser Phe Arg
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ser Leu Ser Ser Arg Gln Ser Pro
1 5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ser Pro Arg Pro Arg Ser Thr Pro
1 5

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Gln Arg Thr Lys Arg Lys His Asn
1 5

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Pro Arg Leu Arg His Pro Arg
1 5

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Asn Leu Arg Lys Arg Lys Ile Lys
1 5

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Asn Ser Thr Val Arg Lys Arg Lys
1 5

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Pro Pro Pro Phe Arg Arg Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Pro Leu Ile Leu Arg Ser Arg Ala
1 5

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Lys Lys Ile Glu Arg Arg Asn Thr
1 5

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
Gln Lys Arg Gly Arg Glu Pro Arg
 1               5
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 8 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
Glu Glu Lys Lys Lys His Lys Lys
 1               5
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 8 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Arg Gln Asn Arg Arg Pro Ser Asn
 1               5
```

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 8 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
Val Arg Pro Ala Arg Ala Leu His
 1               5
```

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 8 amino acids
  (B) TYPE: amino acid
  (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Leu Ile Ala Leu Arg Ser Thr Thr
1 5

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Pro Thr Pro Leu Lys His Pro Arg
1 5

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Pro Tyr Pro Pro Lys Arg Thr Pro
1 5

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Leu Ser Thr Ser Arg Ala Ser Ile
1 5

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Thr Gly Val His Lys Pro Ser Thr
1 5

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Leu Cys Ala Lys Arg Leu Tyr Arg
1 5

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Arg Lys Pro Thr Lys Lys Asn Ser
1 5

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Glu Cys Arg Gln Arg His Thr Arg
1 5

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Ser Leu Ala Leu Arg Val Trp Arg
1 5

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gly Pro Arg Leu Arg His Pro Arg
1 5

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Phe Ile Ser Arg Arg Val Cys Arg
1 5

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Pro Asp Asn Gln Arg Tyr Ile Thr
1 5

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

```
Pro Leu Pro Cys Lys Leu Asp Ala
 1               5
```

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```
Ile Arg Phe Ala Arg Ser Gln Ala
 1               5
```

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

```
Pro Thr Pro Leu Lys His Pro Arg
 1               5
```

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

```
Pro Phe Thr His Lys Ser Leu Ser
```

1 5

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Ser Val Leu Pro Lys Leu Arg Ile
1 5

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Pro Lys Glu Thr Lys Gln Thr Asn
1 5

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Ser Leu Ser Ser Arg Gln Ser Pro
1 5

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

```
Thr Pro Leu Leu Lys Ser Trp Leu
 1               5
```

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

```
Arg Asn Arg Gln Lys Thr Asn Asn
 1               5
```

Claims

1. A peptide having the amino acid sequence:
Arg-Asn-Arg-Gln-Lys-Thr (SEQ.ID NO.1).

2. A peptide selected from the group consisting of:

Arg-Asn-Arg	(SEQ.ID NO.2),
Arg-Asn-Arg-Gln	(SEQ.ID NO.3),
Arg-Asn-Arg-Gln-Lys	(SEQ.ID NO.4),
Asn-Arg-Gln-Lys-Thr	(SEQ.ID NO.5),
Arg-Gln-Lys-Thr	(SEQ.ID NO.6),
Gln-Lys-Thr	(SEQ.ID NO.7),
Arg-Gln-Lys	(SEQ.ID NO.8),
Asn-Arg-Gln	(SEQ.ID NO.9), and
Arg-Gln-Lys	(SEQ.ID NO.10),

3. The peptide of claims 1 or 2, wherein said peptide contains 1, 2, 3, 4, 5, or 6 conservative amino acid substitutions.

4. The peptide of claims 1, 2, or 3, wherein the amino acids are covalently coupled by non-hydrolyzable peptide bonds.

5. The peptide of claims 1,2,3, or 4, said peptide further including a derivatizing agent that covalently binds to an amino acid in the substrate binding site of a mast cell tryptase-6 complex.

6. The peptide of claim 5, wherein the derivatizing agent is present on an amino acid of the peptide selected from the group consisting of:

(a) an N-terminal amino acid of the peptide; and

(b) a C-terminal amino acid of the peptide.

7. A tryptase-6 complex inhibitor that is a functionally equivalent peptide of SEQ.ID NO. 1, said functionally equivalent peptide having the formula:

X-P-Y,

wherein:

P is a peptide selected from the peptides of claims 1,2,3, or 4;

X is an N-terminal peptide containing from zero to five amino acids;

Y is a C-terminal peptide containing from zero to five amino acids;

wherein said functionally equivalent peptide competitively inhibits cleavage of a peptide having SEQ.ID NO. 1 by the tryptase-6 complex.

8. The tryptase-6 complex inhibitor of claim 7,

wherein X contains from zero to five amino acids of the peptide sequence in **fibronectin** that is N-terminal to the fibronectin amino acids 1351-1356; and

wherein Y contains from zero to five amino acids of the peptide sequence in fibronectin that is C-terminal to the fibronectin amino acids 1351-1356.

9. The peptide of claims 1-6 or the tryptase-6 complex inhibitor of claims 7-8, wherein the tryptase-6 complex is a human tryptase-6 complex.

10. A method for selecting a tryptase-6 complex inhibitor comprising:

determining whether a tryptase-6 complex cleaves a peptide that is or that contains the amino acid sequence of SEQ.ID NO. 1 in the presence of a putative protease inhibitor.

11. The method of claim 10, wherein the tryptase-6 complex is a human tryptase-6 complex.

12. The method of claim 10, wherein the tryptase-6 complex is an mMCP-6 complex.

13. The method of claim 10, wherein the putative protease inhibitor is contained in a phage display library.

14. A method for treating a mast cell-mediated inflammatory disorder comprising:

administering to a subject in need of such treatment a peptide of claims 1-6 or a tryptase-6 complex inhibitor of claims 7-9 in a pharmaceutically acceptable carrier and in an amount effective to inhibit activity of a tryptase-6 complex in said subject.

INTERNATIONAL SEARCH REPORT

Inter ...nal Application No
PCT/US 98/01865

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K07/06 C07K07/08 C07K14/81 C07K5/08 C07K5/10
A61K38/08 A61K38/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BAJUSZ S: "INTERACTION OF TRYPSIN-LIKE ENZYMES WITH SMALL INHIBITORS" SYMPOSIA BIOLOGICA HUNGARICA, vol. 25, 1 January 1984, pages 277-298, XP000560985 see the whole document ---	3,4,7-9, 14
X	HERSHKOVIZ R ET AL: "NONPEPTIDIC ANALOGUES OF THE ARG-GLY-ASP (RGD) SEQUENCE SPECIFICALLY INHIBIT THE ADHESION OF HUMAN TENON'S CAPSULE FIBROBLASTS TO FIBRONECTIN" INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE, vol. 35, no. 5, April 1994, pages 2585-2591, XP000616130 see the whole document see figure 1; table 2 ---	3,4,7-9, 14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 May 1998	24 06 1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cervigni, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STEINER B ET AL: "PEPTIDES DERIVED FROM A SEQUENCE WITHIN B3 INTEGRIN BIND TO PLATELET AIIBB3 (GPIIB-IIIA) AND INHIBIT LIGAND BINDING" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 10, 5 April 1993, pages 6870-6873, XP000354929 see table 1 ---	3,4,7-9, 14
X	WO 95 21861 A (MERCK & CO INC ;WIEDERRECHT GREGORY J (US); SEWELL TONYA J (US)) 17 August 1995 see page 9, line 19 ---	3,4,7-9, 14
X	US 5 187 157 A (KETTNER CHARLES A ET AL) 16 February 1993 cited in the application see the whole document ---	5,6
A	J. LOHI ET AL: "Pericellular substrates of human mast cell tryptase: gelatinase and fibronectin" J. CELLULAR BIOCHEMISTRY, vol. 50, no. 4, December 1992, pages 337-349, XP002064383 see the whole document see page 347, column 1 -----	

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/01865

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claim 14 is directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten nal Application No
PCT/US 98/01865

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9521861 A	17-08-1995	US 5457182 A	10-10-1995
		CA 2181803 A	17-08-1995
		EP 0749443 A	27-12-1996
		JP 9509160 T	16-09-1997
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		PT 87652 B	30-09-1992
		US 5242904 A	07-09-1993
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		SU 1807988 A	07-04-1993
		RU 2017749 C	15-08-1994